3-4-91

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

003235

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: SAN 582H: Experimental Use Permit

Caswell No. 195J HED Project No. 0-1937A

TO: Sangeeta Vohra
Product Manager 23
Registration Division (H7505C)

FROM: Deborah L. McCall 2-14-91
HFAS / HED / Toxicology Branch II / (H7509C)

THROUGH: James Rowe, Ph.D., Section Head James Rowe 2/14/91
Section III/HFAS/Toxicology Branch II/HED (H7509C)
and
Marcia Van Gemert, Ph.D., Branch Chief Marcia Van Gemert 3/4/91
~~HFAS / HED / Toxicology~~ Branch II / (H7509C)

The registrant (Sandoz Crop Protection Corporation) has submitted 21 studies in support of an Experimental Use Permit for [redacted] and for a petition for corn (0.1 ppm). These studies have been evaluated, and the Data Evaluation Reports are attached. The conclusions are as follows:

SAN 582H TECHNICAL:

1) Acute Oral in Rats (§81-1) (MRID No. 416624-09) - The study was classified Guideline. LD50 = 2139.8 mg/kg in males and 1296.8 mg/kg in female rats. The combined LD50 = 1569.8 mg/kg. Toxicity Category III.

2) Acute Dermal in Rats (§81-2) (MRID No. 416624-10) - The study was classified as Guideline. LD50 > 2.0 mg/kg in both sexes. Toxicity Category III.

3) Acute Inhalation in Rats (§81-3) (MRID No. 416624-11) - The study was classified as Minimum. LC50 > 4990 mg/m^3 in male and female rats. Toxicity Category III.

4) Primary Eye Irritation in Rabbits (§81-4) (MRID No. 416624-12) - The study was classified as Minimum. Minimally irritating to the eyes of white rabbits. Toxicity Category III.

5) Primary Dermal Irritation in Rabbits (§81-5) (MRID No. 416624-13) -The study was classified as Guideline. Minimally irritating to the skin of male rabbits. Toxicity Category IV.

6) Dermal Sensitization in Guinea Pigs (§81-6) (MRID No. 417068-07) - The study was classified as Minimum. SAN 582H is a mild skin sensitizer in male guinea pigs.

7) 13-Week Oral Feeding Study in Rats (§82-1) (MRID No. 416159-01) - The study was classified as Minimum. The oral NOEL = 500 ppm and the LOEL = 1500 ppm. Compound related reductions in body weight, increase in relative liver weight, centrilobular hepatocytic enlargement and an increase in protein and cholesterol levels were observed at 1500 ppm or higher dose levels.

8) 13-Week Oral Feeding Study in Dogs (§82-1)(MRID No. 416159-02) - The study was classified as Minimum. NOEL = 100 ppm and the LOEL = 750 ppm. Periportal hepatocellular vacuolation was noted in the 2000 ppm (HDT) dose group. Histological liver changes were noted in the 750 and 2000 ppm dose group. Body weight and body weight gains of the 750 ppm (females only) and the 2000 ppm (males only) were depressed throughout the study.

9) 52-Week Oral Toxicity Study in Dogs (§83-1) (MRID No. 416159-03) - The study was classified as Guideline. NOEL = 250 ppm and the LOEL = 1250 ppm. Periportal hepatocyte vacuolation was noted in the 1250 ppm dose group. Liver changes in the 1250 ppm dose group correlates with the increase in serum alkaline phosphatase, cholesterol levels, and the increase in liver-to-body weight ratio.

10) Developmental study in Rats (§83-3) (MRID No. 416159-04) - The study was classified as Minimum. Maternal NOEL = 50 mg/kg/d and the LOEL = 215 mg/kg/d. Maternal toxicity was evidenced by excess salivation, increased liver weights and reduced body weight gain and food consumption in the 215 and 425 mg/kg (HDT). Developmental toxicity was evidenced by increased incidence of resorptions in the 425 mg/kg dose group. Developmental NOEL = 215 mg/kg/d and the LOEL = 425 mg/kg/d.

11) Two Generation Reproduction in Rats (§83-4) (MRID No. 416159-05) - The study was classified as Minimum. Parental toxicity NOEL = 500 ppm and the LOEL = 2000 ppm. Parental toxicity was evidenced by significant reductions in body weight and food consumption in males and significant increase in absolute and relative liver weights in both sexes of the 2000 ppm dose group. Significant reductions in pup weight during lactation were noted in the 2000 ppm dose group. The NOEL and LOEL for reproductive toxicity were 500 and 2000 ppm, respectively.

12) Salmonella/mammalian reverse activation gene mutation assay (§84-1) (MRID No. 415965-42) - The study was classified as Acceptable. No increase in mutant colonies of any strain at any dose either with or without S9 activation were noted.

13) In vitro Chromosome Aberration using Chinese hamster ovary (§84-2) (MRID No. 415965-43) - The study was classified as Unacceptable. Cytotoxicity was apparent at non-activated dose level of 125 to 150 µg/mL and S9-activated levels of 400 to 500 µg/mL. Study should be repeated at dose levels well below the cytotoxic dose.

14) Rat Primary Unscheduled DNA (in vitro) synthesis (§84-3)(MRID No. 415965-44) - The study was classified as Acceptable. Genotoxic (unequivocally positive), UDS activity occurred at levels well below the cytotoxic level.

15) Metabolism in Rats (§85-1) (MRID No. 415965-45) - The study was classified as Unacceptable. SAN 582H was extensively metabolized within 3 days after dosing. Less than 2.5% of the ^{14}C dose was recovered as unchanged parent compound, and 22 metabolites, 21 were found in the urine and feces and were identified. However, because 61 to 78% of the ^{14}C dose was not identified or characterized the study is unacceptable.

SAN 582H 7.5L:

1) Acute Oral Toxicity in Rats (§81-1) (MRID No. 415965-36) - The study was classified as Guideline. The LD50 = 2.4 g/kg for combined sexes. The male LD50 = 2.0 g/kg and the female LD50 = 2.8 g/kg. Clinical signs included: hypoactivity, clonic convulsions, tremors, ataxia, excess salivation, diarrhea, red-stained face and yellow-stained urogenital area. Toxicity Category III.

2) Acute Dermal Toxicity in Rabbits (§81-2) (MRID No. 415965-37) - The study was classified as Guideline. LD50 > 2.0 g/kg for combined sexes. No deaths occurred during the study. Clinical signs included: severe dermal irritation consisting of slight to severe erythema, atonia, and fissuring. No systemic toxicity was noted. Toxicity Category III.

3) Acute Inhalation Study in Rats (§81-3) (MRID No. 415965-38) - The study was classified as Minimum. LC50 > 3.39 mg/L. No deaths occurred during the study. Clinical signs included: partial closing of eyes, salivation, reduced respiratory rate and abnormal body position during exposure. After exposure the signs were: Ataxia, abnormal respiration, ocular discharge, and lethargy. Toxicity Category III.

4) Primary Eye Irritation in Rabbits (§81-4) (MRID No. 415965-39) - The study was classified as Guideline. Slight to severe conjunctival irritation, slight corneal opacity and iritis were observed up to day 7. All eyes were normal by day 21. Toxicity Category II.

5) Primary Dermal Irritation in Rabbits (§81-5) (MRID No. 415965-40) - The study was classified as Guideline. Primary Dermal Irritation score = 0.6, Slightly irritating. Slight to well-defined erythema was noted up to 72 hrs. and slight edema was noted up to 48 hrs. post exposure. No irritation was observed 96 hrs. post exposure. Toxicity Category IV.

6) Dermal Sensitization in Guinea Pigs (§81-6) (MRID No. 415965-41) - The study was classified as Guideline. SAN 532H is a dermal sensitizer, eliciting slight to moderate dermal reactions.

RECOMMENDATION: [redacted]

Concerning the "G" Petition - temporary tolerance for Corn (0.1 ppm) the TOX II branch has possible carcinogenicity concerns due to positive mutagenicity data. Please submit data which will address the chronic concerns.

PENDING REGISTRATION INFORMATION IS NOT INCLUDED

068285

Reviewed by: Timothy F. McMahon, Ph.D. 2/5/91
Section I, Toxicology Branch II (HFAS) (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 2/5/91
Section I, Toxicology Branch II (HFAS) (H7509C)

Data Evaluation Report

Study type: Acute oral-rats (81-1) Tox. Chem. No.: 195J

MRID number: 416624-09

Test material: SAN 582H Technical

Study number: HLA 686-171

Testing Facility: Hazelton Laboratories America, Inc.
Vienna, Virginia 22180

Sponsor: Sandoz Crop Protection Corporation
Des Plaines, Illinois

Title of report: Acute Oral Toxicity Study in Rats with SAN 582H Technical

Author(s): Joan K. Lemem, M.S.

Report issued: January 11, 1989

Conclusions:

Under the conditions of this study, the acute oral LD_{50} of SAN 582H Technical was 2139.8 mg/kg in male rats, with 95% confidence limits of 1444.9 and 3163.9 mg/kg body weight. The estimated oral LD_{50} in female rats was calculated as 1296.8 mg/kg body weight, with 95% confidence limits of 898.7 and 1871.5 mg/kg body weight. The combined oral LD_{50} was calculated as 1569.8 mg/kg, with 95% confidence limits of 1174.3 and 2098.3 mg/kg body weight.

When calculated using the probit method, the LD_{50} for SAN 582H Technical was 1501.3 mg/kg for male and female rats combined, with 95% confidence limits of 1221.7 and 1844.8 mg/kg body weight.

Toxicity Catgory III

Core Classification: guideline

This study fulfills the requirements (81-1) for an acute oral toxicity study in rats.

I. MATERIALS

A. Test Material: SAN 582H Technical; description: dark brown liquid; purity: 91.4%. lot no. 8605

B. Test Animals: Male and female Sprague-Dawley (Hsd:BR) rats; Source: Harlan Sprague-Dawley, Frederick, MD. Age: young adult. Weight: males: 217-239g; females, 211-239g.

II. METHODS

Five male and 5 female rats per dose group were selected for use in this study. Rats were given food (Purina Certified Rodent Chow #5002) and tap water *ad libitum*, with the exception of an overnight fast prior to dosing . Rats were acclimated for approximately 2 weeks prior to test article administration. Rats were housed individually in temperature and humidity controlled rooms. A 12 hour light/dark cycle was used.

Five male and 5 female rats were selected per dose group for dosing using computer generated random numbers. Separate groups of rats were administered single oral doses of 1000, 2000, or 3000 mg/kg body weight of SAN 582H Technical in corn oil in a dose volume of 10 ml/kg. Each animal was observed for signs of clinical toxicity and pharmacotoxicity at 1, 2, and 4 hours following test article administration, and once daily thereafter. Mortality and/or moribundity were recorded twice daily. Body weights were recorded immediately prior to test article administration, on day 7 following test article administration, and at animal termination (day 14) or time of death. Animals were killed on day 14 by CO_2 inhalation and were subjected to gross necropsy

Calculation of the median lethal dose (LD_{50}) and 95% confidence intervals were estimated for both males and females as well as males and females combined using a modified Behrens-Reed-Muench method. Data were re-analyzed for males and females combined using the probit method in order to determine the slope of the dose response curve of the estimated LD_{50} (Amendment I, page 21 of report).

III. RESULTS

Mortality

At the 1000 mg/kg dose level, one female rat was found dead on day 2 post dosing. No mortality was observed in males at this dose level.

At the 2000 mg/kg dose level, 2 males were found dead on day 1 following dosing, and one male was found dead on day 5 following dosing. For females at this dose level, 2 were found dead on day 1 following dosing, 1 was found dead on day 2 following dosing, and the remaining 2 in this group were found dead on day 10 following dosing.

At the 3000 mg/kg dose level, 2 males were found dead on day 1 following dosing, 2

were found dead on day 2 after dosing, and the remaining 1 rat was found dead on day 4 after dosing. In females at this dose level, 1 animal was found dead 4 hours after dosing, and the remaining 4 were found dead on day 1 after dosing.

Clinical Toxicity

At 1000 mg/kg, no signs were observed in male rats. In female rats, slight depression was observed in 1 female rat from 1-4 hours after dosing, and in 1 other rat from 2-4 hours after dosing.

At 2000 mg/kg, a variety of clinical signs were observed in both males and females, the most prevalent being slight depression (4 of 5 males from 1-4 hours post treatment, 5 of 5 females from 1-4 hours post treatment). Other signs (prostration, tremors, convulsions, red staining of the nose and/or eyes) ataxia, rough coat) occurred in 1 to 2 rats per sex in this dose group during the first 10 days after treatment. While stated on page **16** that slight depression and soft feces were the only toxic signs observed in surviving rats at this dose, there is no indication of this in the Table on page **12** of the report.

At 3000 mg/kg, all rats were dead by day 5 after treatment. Slight depression was again the most prevalent toxic sign in both males and females during the first 4 hours following treatment. Prostration and red staining of the nose and/or eyes was observed in a few male rats from days 1-3 after dosing.

Macroscopic examination of rats at necropsy showed mottling of the lungs, mottling and discoloration of the liver and spleen, discoloration of the kidneys, and abnormal contents of the stomach and intestines in treated males and females.

Body weight gain was not significantly affected at the 1000 mg/kg dose level in male or female rats. At the 2000 mg/kg dose level, some weight loss was evident in male rats, but was not statistically significant. In female rats, weight loss was more apparent, and was statistically significant, although all female rats died before the 14 day post-treatment period. At the 3000 mg/kg dose level, a similar pattern of weight loss occurred for both male and female rats as was observed at the 2000 mg/kg dose level.

Median Lethal Dose Estimation

The estimated oral LD_{50} in male rats was calculated as 2139.8 mg/kg with 95% confidence limits of 1444.9 and 3168.9 mg/kg body weight. The estimated oral LD_{50} in female rats was calculated as 1296.8 mg/kg body weight, with 95% confidence limits of 898.7 and 1871.5 mg/kg body weight. The combined oral LD_{50} was calculated as 1569.8 mg/kg, with 95% confidence limits of 1174.3 and 2098.3 mg/kg body weight.

When calculated using the probit method, the LD_{50} for SAN 582H Technical was 1501.3 mg/kg for male and female rats combined, with 95% confidence limits of 1221.7 and 1844.8 mg/kg body weight.

IV. CONCLUSIONS

Under the conditions of this study, the acute oral LD_{50} of SAN 582H Technical was 2139.8 mg/kg in male rats, with 95% confidence limits of 1444.9 and 3168.9 mg/kg body weight. The estimated oral LD_{50} in female rats was calculated as 1296.8 mg/kg body weight, with 95% confidence limits of 898.7 and 1871.5 mg/kg body weight. The combined oral LD_{50} was calculated as 1569.8 mg/kg, with 95% confidence limits of 1174.3 and 2098.3 mg/kg body weight.

When calculated using the probit method, the LD_{50} for SAN 582H Technical was 1501.3 mg/kg for male and female rats combined, with 95% confidence limits of 1221.7 and 1844.8 mg/kg body weight.

Toxicity Category III

V. CORE CLASSIFICATION: guideline

This study fulfills the requirements (81-1) for an acute oral toxicity study in rats.

008285

Reviewed by: Timothy F. McMahon, Ph.D. 2/5/91
Section I, Toxicology Branch II (HFAS) (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 2/5/91
Section I, Toxicology Branch II (HFAS) (H7509C)

Data Evaluation Report

Study type: Acute dermal-rabbits (81-2) Tox. Chem. No.: 195J

MRID number: 416624-10

Test material: SAN 582H Technical

Study number: HLA 686-172

Testing Facility: Hazelton Laboratories America, Inc.
Vienna, Virginia 22180

Sponsor: Sandoz Crop Protection Corporation
Des Plaines, Illinois

Title of report: Acute Dermal Toxicity Study In Rabbits with SAN 582H Technical

Author(s): Joan K. Lemen, B.S.

Report issued: December 16, 1988

Conclusions:

Under the conditions of this study, the acute dermal LD_{50} of SAN 582H Technical was > 2.0g/kg in male and female rabbits.

Toxicity Catgory III

Core Classification: guideline

This study fulfills the requirements (81-2) for an acute dermal toxicity study in rabbits.

I. MATERIALS

A. Test Material: SAN 582H Technical; description: dark brown liquid; purity: 91.4%. Lot # 8605

B. Test Animals: Male and female Hra: (NZW) SPF rabbits; Source: Hazelton Research Products, Inc., Denver, PA. Age: young adult. Weight: males: 2.0 kg; females, 2.0 kg.

II. METHODS

Five male and 5 female rats were selected at random using a computer randomization procedure. Rabbits were given 120g of food per day (Purina Certified High Fiber Rabbit Chow # 5325) and tap water *ad libitum*. Rabbits were acclimated to the lab environment for at least 7 days prior to start of the study. Rabbits were housed individually in temperature and humidity controlled rooms in stainless steel cages.

Approximately 24 hours prior to application of the test material, rabbits were shaved on the dorsal surface from shoulder to rump. Test material was then applied as received at a dose level of 2 g/kg to a 20 x 13 cm area of shaved intact skin using a glass rod. No adjustment was made in dosing for the purity of the compound (91.4%). Rubber damming was placed around the trunk of each rabbit and secured in place with porous adhesive tape. Immediately after application of test material, rabbits were fitted with collars to prevent ingestion of test material. Duration of exposure to test material was 24 hours.

Twenty-four hours after application, binders were removed and the skin wiped with water moistened gauze. Collars remained on the rabbits until the day of sacrifice due to the large amount of test material remaining on the skin.

Rabbits were observed for clinical toxicity and mortality immediately after test material application, and then at least once daily for the remainder of the 14 day test period. Skin reaction to application of test material was graded on days 1, 3, 7, 10, and 14 according to the Draize method.

On day 14, surviving rabbits were euthanized by sodium pentobarbital injection and subjected to gross necropsy. All gross lesions in rabbits surviving 24 hours or longer were saved in 10% buffered formalin.

III. RESULTS

No mortality or signs of clinical toxicity were observed in either male or female rabbits during the study period. Slight body weight loss was observed in 4 of 5 male rabbits and 5 of 5 female rabbits between days 0 (day of exposure) and day 7. However, weight gain was observed in these same rabbits from test days 7-14. Staining of the skin site with test material was observed in all rabbits at necropsy, and small testes (bilateral) were observed in 1 male rabbit.

Slight erythema was observed in 3 of 5 male rabbits and 1 of 5 female rabbits on day 1 following application of test material. Well-defined erythema was observed in 2 of 5 male rabbits and 1 of 5 female rabbits on day 1 following test material application. No sign of erythema was observed in any rabbit after day 7 following test material application.

No signs of edema were observed in any rabbit over the duration of the study. However, brown staining of the application site was observed in all rabbits, and epidermal scaling was observed in 3 rabbits on days 7 and 14.

IV. CONCLUSIONS

Under the conditions of this study, the acute dermal LD_{50} of SAN 582H technical was > 2.0 g/kg in male and female rabbits.

Toxicity Category III

V. CORE CLASSIFICATION

guideline

This study fulfills the requirements (81-2) for an acute dermal toxicity study in rabbits.

008285

Reviewed by: Timothy F. McMahon, Ph.D. T.F.M. 2/5/91
Section I, Toxicology Branch II (HFAS) (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Y.M.I. 2/5/91
Section I, Toxicology Branch II (HFAS) (H7509C)

Data Evaluation Report

Study type: Acute inhalation-rats (81-3) Tox. Chem. No.: 195J

MRID number: 416624-11

Test material: SAN 582H Technical

Study number: RCC 075510

Testing Facility: Research and Consulting Company AG
CH-4452 Itingen, Switzerland

Sponsor: Sandoz Crop Protection Corporation
Des Plaines, Illinois

Title of report: 4-Hour Acute Inhalation Toxicity Study With SAN 582H In Rats

Author(s): L. Ullmann

Report issued: September 19, 1986

Conclusions:

Under the conditions of this study, the acute inhalation LC_{50} of SAN 582H Technical was > 4990 mg/m^3 in male and female rats.

Toxicity Catgory III

Core Classification: minimum
This study satisfies the guideline requirements (81-3) for an acute inhalation toxicity study in rats.

I. MATERIALS

A. Test Material: SAN 582H; description: brown oil; purity: 91.4%; batch # 8605

B. Test Animals: Five male and 5 female KFM-HAN Wistar rats; Source: Kleintierfarm Madoerin AG, Fuellinsdorf/Switzerland. Age (at exposure): 12-14 weeks. Weight: males: 259-286g; females, 215-243g.

II. METHODS

A. Animal Husbandry

Five male and 5 female rats were selected at random from a batch of rats for use in this study based on body weight and health status. Rats were given food (Pelleted standard Kliba 343 rat maintenance diet ("Kliba", Klingentalmuehle AG, 4303 Kaiseraugst, Switzerland) and community tap water *ad libitum*. Rats were acclimated to the lab environment for at least one week following veterinary examination. Rats were housed in prior to exposure in groups of five in Makrolon type 4 cages with standard softwood bedding. Rats were housed under conditions of controlled temperature (22± 3 °C) and humidity (40-70%). A 12 hour light/dark cycle was used.

B. Atmosphere Generation

Test material was heated to 45 °C and supplied to a nozzle by an infusion pump (Perfusor VI ED/300) into a high velocity air stream, which discharged into the exposure chamber. Air flow was stated as 600 L/hr. Chamber air flow rate was monitored throughout the exposure period, but a range and mean air flow was not provided. A diagram of the exposure apparatus was provided (page 31 of report).

C. Exposure

Rats were subjected to nose-only exposure to test material in separate polyvinylchloride tubes surrounding an approximately 100 liter polyvinylchloride nose-only chamber (Appendix C, page 31 of report). Exposure duration was for 4 hours to test material.

Chamber concentrations of test material were performed gravimetrically on five separate occasions using selectron filters of 0.2 µm pore size (location of measurement not stated). Particle size distribution was assessed using an eight stage Andersen Ambient Particle Sizing Sampler two times during exposure. Aerodynamic mass median diameter and geometric standard deviation were apparently not determined.

Oxygen content, humidity, and temperature were measured eight times during exposure.

D. Post-Exposure Observations

Animals were observed for toxic signs and mortality four times on the day of exposure, and daily thereafter until day 15. Body weights were recorded on day 1 (day of exposure) and days 8 and 15 post exposure. All survivors to day 15 were euthanized by intraperitoneal injection of sodium pentobarbitone and subjected to gross necropsy.

III. RESULTS

A. Atmosphere Generation

Measured chamber concentration of test material over the exposure period averaged 4990 ± 84mg/m^3 (range: 4870-5080 mg/m^3). Aerodynamic mass median diameter was not provided. It was stated in the report (page 9) that 17.5% of the particles were in an inhalable range of 0.4-5.8μm. (Appendix C, page 32 of report).

B. Animal Observations

No mortality was observed during exposure or during the post-exposure observation period. Sedation, dyspnea, ruffled fur, and hunched body posture was observed in 3 of 5 male and female rats prior to the 4 hour termination period for exposure. Symptoms continued in both sexes until test day 3 (2 of 5 rats with symptoms at 24 hours, 1 of 5 rats with symptoms at 3 days). Symptoms occurred with equal frequency and duration in both sexes. Over the duration of the experiment, male rats gained approximately 9% of initial body weight, while female rats gained approximately 2% of initial body weight. Net loss of body weight was observed in 1 female rat on day 8 of the experiment, and in 1 female rat on day 8 and 15. However, the loss of weight was insignificant.

At necropsy, discoloration and/or hemorrhage was noted in the lungs of all but 3 rats. No other gross pathologic findings were reported.

IV. CONCLUSIONS

Under the conditions of this study, the acute inhalation LC_{50} of SAN 582H Technical was > 4990mg/m^3 in male and female rats. Although the percentage of particles with a size of 1μm or less was approximately 10.4% (which does not meet the recommended percentage of 25% of particles with a size of 1μm or less for acute inhalation exposures), this study is considered acceptable based on the fact that this chemical demonstrated very low acute toxicity and the fact that any exposure to the technical grade of SAN 582H wouold be very limited.

Toxicity Category III

V. CORE CLASSIFICATION

minimum

This study satisifes the guideline requirements (81-3) for an acute inhalation toxicity study in rats.

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Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 2/5/91
Section I, Toxicology Branch II (HFAS) (H7509C)

Data Evaluation Report

Study type: Primary eye irritation-rabbits (81-4) Tox. Chem. No.: 195J

MRID number: 416624-12

Test material: SAN 582H technical

Study number: HLA 686-174

Testing Facility: Hazelton Laboratories America, Inc.
Vienna, Virginia 22180

Title of report: Primary Eye Irritation Study in Rabbits with SAN 582H Technical

Author(s): Joan K. Lemen, B.S.

Report issued: November 30, 1988

Conclusions:

Under the conditions of this study, SAN 582H technical was determined to be minimally irritating to the eyes of white rabbits.

Toxicity Catgory III

Core Classification: minimum

This study satisfies the requirements (81-4) for a primary eye irritation study in rabbits.

I. MATERIALS

A. Test Material: SAN 582H Technical; description: dark brown liquid; purity: 91.4%. Lot # 8605

B. Test Animals: Male and female New Zealand White Rabbits (Hra: (NZW) SPF strain); Source: Hazelton Research Products, Inc., Denver, PA. Age: young adult. Weight: not provided.

II. METHODS

Six rabbits (three male and three female) were employed for study of the primary eye irritation of SAN 582H Technical. Rabbits were randomized at receipt by use of computer generated random numbers. Rabbits were given 120g of food per day (Purina Certified High Fiber Rabbit Chow # 5325) and tap water *ad libitum*. Rabbits were acclimated to the lab environment for at least 7 days prior to start of the study. Rabbits were housed individually in temperature and humidity controlled rooms in screen-bottomed cages.
Note: The automated watering system used in this study was inoperable for one day during acclimation of the rabbits. Water bottles were provided during this period.

Approximately 24 hours prior to application of test material, the left and right eye of each rabbit was examined using sodium fluorescein dye. Only those rabbits with no sign of ocular injury or irritation were used.

SAN 582H Technical (0.1ml) was placed on the everted lower lid of the left eye of all rabbits, with the right eye serving as untreated control. Upper and lower lids were gently held together for 1 second to prevent loss of test material. Treated eyes were not washed.

Irritation to the cornea, iris, and conjunctiva was evaluated at 1, 24, 48, and 72 hours after instillation of the test material according to the Draize scale. All rabbits were sacrificed by injection of sodium pentobarbital and penetration of the thoracic cavity at study termination, and discarded without necropsy.

III. RESULTS

No corneal opacity or iritis was observed in any of the rabbits at any time of observation following administration of test material. Mild redness of the conjunctiva was observed in all rabbits 1 hour following application of test material, but was not evident 72 hours after treatment in any rabbit. Minimal chemosis was observed in 4 of 6 rabbits at 1 hour following application of test material, and minimal discharge was observed in all rabbits at 1 hour post-treatment. No signs of chemosis or discharge were evident in any rabbit at 72 hours following treatment.

IV. CONCLUSIONS

Under the conditions of this study, SAN 582H technical was determined to be minimally irritating to the eyes of white rabbits.

Toxicity Category III

V. CORE CLASSIFICATION

minimum

This study satisfies the requirements (81-5) for a primary eye irritation study in rabbits.

008285

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Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. JMI 2/5/91
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Data Evaluation Report

Study type: Primary dermal-rabbits (81-5) Tox. Chem. No.: 195J

MRID number: 416624-13

Test material: SAN 582H Technical

Study number: HLA 686-173

Testing Facility: Hazelton Laboratories America, Inc.
Vienna, Virginia 22180

Sponsor: Sandoz Crop Protection Corporation
Des Plaines, Illinois

Title of report: Primary Dermal Irritation Study In Rabbits with SAN 582H Technical

Author(s): Joan K. Lemen, B.S.

Report issued: November 30, 1988

Conclusions:

Under the conditions of this study,SAN 582H Technical was minimally irritating to the skin of male rabbits.

Toxicity Catgory IV

Core Classification: guideline

This study fulfills the requirements (81-5) for a primary dermal irritation study in rabbits.

I. MATERIALS

A. Test Material: SAN 582H Technical; description: dark brown liquid; purity: 91.4%.
Lot # 8605

B. Test Animals: Male and female Hra: (NZW) SPF rabbits; Source: Hazelton Research Products, Inc., Denver, PA. Age: young adult. Weight: not stated.

II. METHODS

Six acclimated male rabbits were selected at random using a computer randomization procedure. Rabbits were given 120g of food per day (Purina Certified High Fiber Rabbit Chow # 5325) and tap water *ad libitum*. Rabbits were acclimated to the lab environment for at least 11 days prior to start of the study. Rabbits were housed individually in temperature and humidity controlled rooms in screen-bottomed cages with a 12 hour light/dark cycle.

Approximately 24 hours prior to application of the test material, hair was clipped from the backs and flanks of each rabbit and an application site chosen. A 0.5ml aliquot of undiluted test material was then applied to the dorsal skin approximately 6cm^2 in area. No adjustment was made in dosing for the purity of the compound (91.4%). Application sites were covered with a 2-inch square gauze patch secured with transparent tape. Rubber damming was then placed around the trunk of each rabbit and secured in place with porous adhesive tape. Rabbits were then fitted with collars to prevent ingestion of test material. Duration of exposure to test material was 4 hours.

Four hours after application of test material, the patches, binders, and collars were removed and the application sites wiped with water moistened gauze to remove residual test material. The degree of erythema and edema was assessed 30 to 60 minutes following removal of test material, and again at 24, 48, and 72 hours using the Draize method.

At study termination, all rabbits were sacrificed by sodium pentobarbital injection and penetration of the thoracic cavity. Rabbits were discarded without necropsy.

III. RESULTS

No mortality or signs of clinical toxicity were observed in either male or female rabbits during the study period.

Slight erythema was observed in 4 of 6 rabbits at the 30-60 minute observation time. However, no signs of erythema were reported by 24 hours. Slight edema was also observed in 1 rabbit at the 30-60 minute observation period, but no signs of edema were present in any rabbit at the 24 hour observation period.

IV. CONCLUSIONS

Under the conditions of this study, SAN 582H Technical was minimally irritating to the skin of male rabbits.

Toxicity Category IV

V. CORE CLASSIFICATION

guideline

This study fulfills the requirements (81-5) for a primary dermal irritation study in rabbits.

Reviewed by: Timothy F. McMahon, Ph.D. 3/9/91
Section I, Toxicology Branch II (HFAS) (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 3/9/91
Section I, Toxicology Branch II (HFAS) (H7509C)

008285

Data Evaluation Report

Study type: Dermal sensitization-guinea pigs (81-6) Tox. Chem. No.: 195J

MRID number: 417068-07

Test material: SAN 582H Technical

Study number: CBK I. 6501/86

Testing Facility: Sandoz Ltd.
Basle, Switzerland

Sponsor: Sandoz Crop Protection Corporation
Des Plaines, Illinois

Title of report: SAN 582H Skin Sensitization Test in Guinea Pigs

Author(s): F. Hamburger

Report issued: March 26, 1987

Conclusions:

An increase in intensity of dermal reaction to application of SAN 582H was not observed between negative control and test article treated guinea pigs. However, the frequency of response at challenge exposure was higher in test article treated guinea pigs (79%) than in negative controls (45%), indicating that SAN 582H is a mild skin sensitizer in male guinea pigs under the conditions of this study.

Core Classification: minimum

This study satisfies the guideline requirements (81-6) for a dermal sensitization study in guinea pigs.

0022

I. MATERIALS

A. Test Material: SAN 582H; description: dark brown liquid; purity: 91%; lot # 8502; stability: at least two years at room temperature.

B. 1) Positive Control Material: DNCB (1-chloro-2,4 dinitrobenzene)

2) Negative (solvent) Control: dimethylsulfoxide (DMSO)

C. Test Animals: Male guinea pigs (DUHA strain); Source: KFM, Fullinsdorf, Switzerland. Age: approximately 4 weeks; Weight: 200-280g (mean: 235g).

II. METHODS

General:

Guinea pigs were acclimated to the laboratory environment for 8 days prior to the start of the study. Animals had free access to food (KLIBA no. 24-342, Klingentalmuhle AG., Basle) and tap water. Animals were housed in a temperature (23 ± 2 °C) and humidity (30-70%) controlled animal room. A 12 hour light/dark cycle was used. Prior to the main study, a preliminary study was performed with dilutions of 1% and 5% SAN 582H in order to identify the "skin tolerance" (page 9 of report). Based upon these preliminary studies, a concentration of 5% SAN 582H in DMSO was selected for use in the main study for both the induction and challenge phases of the study.

Induction:

Twenty-four hours prior to induction, skin on the dorsal surface of each guinea pig was clipped free of hair (location of clipping and device used to clip hair not provided). Twenty-four hours after clipping, duplicate intradermal injections of 0.1ml of the appropriate test material were made as follows:

Group	Animal Number	Induction	Optimization (Day 8)	Challenge (Day 22)
negative control	1-20	A. adjuvant alone B. DMSO alone C. adjuvant alone	DMSO only	SAN 582H, 5% in DMSO
positive control	1-20	A. adjuvant alone B. 0.1% DCNB in ethanol C. 0.1% DCNB in adjuvant	1% DCNB in ethanol	1% DCNB in ethanol

(continued)

Group	Animal Number	Induction	Optimization (Day 8)	Challenge (Day 22)
test group	101-120	A. adjuvant alone B. SAN 582H 5% in DMSO C. SAN 582H 5% in adjuvant	SAN 582H 5% in DMSO	SAN 582H 5% in DMSO

A,B, and C refer to distinct injection positions on the dorsal surface of the guinea pig.

Optimization:

Animals were clipped free of hair 24 hours prior to challenge exposure and the treatment site treated with 10% Na lauryl sulfate to induce irritation. A patch containing 3ml of the appropriate solution (as outlined above) was affixed over the treatment area with impermeable plastic tape for 48 hours.

Challenge:

A new skin site was shaved, and a patch containing 3ml of the appropriate test substance was applied over the area with impermeable plastic tape for 24 hours.

No criteria were provided for judging whether a test animal was considered to have evidence of dermal sensitization. Evaluation of skin reactions were made according to the following scale:

0=no reaction
1=scattered mild redness
2=moderate and diffuse redness
3=intense redness and swelling

Skin reactions were scored at the optimization phase of the study (day 10) and at the challenge phase of the study (days 23, 24, and 25).

III. RESULTS

In negative control guinea pigs, 9 of 20 animals displayed scattered mild redness at the 72 hour observation time point, a frequency of 45%. A reaction of similar intensity was observed in test article treated guinea pigs at 72 hours, but the frequency was increased (15 of 19 animals; 79%). One animal in the test group died on day 10 of the study, but no data were provided as to cause of death or contributory factors. No dermal reaction was observed in negative control or test group animals at the 24 and 48 hour observation time points.

In guinea pigs treated with positive control material, reactions indicative of moderate to intense redness and swelling were observed in all animals at the 72 hour observation time point, indicating that the method employed in this assay was functioning well.

IV. CONCLUSIONS

An increase in intensity of dermal reaction to application of SAN 582H was not observed between negative control and test article treated guinea pigs. However, the frequency of response at challenge exposure was higher in test article treated guinea pigs (79%) than in negative controls (45%), indicating that SAN 582H is a mild skin sensitizer in male guinea pigs under the conditions of this study.

V. CORE CLASSIFICATION

minimum

This study satisfies the guideline requirements (81-6) for a dermal sensitization study in guinea pigs.

008235

EPA No.: 68D80056
DYNAMAC No.: 337-A
TASK No.: 3-37A
January 4, 1991

DATA EVALUATION RECORD

SAN 582H

Acute Oral Toxicity Study in Rats

STUDY IDENTIFICATION: Glaza, S. M. Acute oral toxicity study of SAN 582H 7.5L in rats. (Unpublished study No. HLA 9100347 conducted by Hazleton Laboratories America, Inc., Madison, WI, and submitted by Sandoz Crop Protection Corp., Des Plaines, IL; dated February 16, 1990). MRID No. 415965-36.

APPROVED BY:

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

Signature: [signature]

Date: 1-3-91

0023

1. CHEMICAL: SAN 582H 7.5L.

2. TEST MATERIAL: SAN 582H 7.5L, batch No. 5032-94, contained 76.2% active ingredient and was described as a brown liquid.

3. STUDY/ACTION TYPE: Acute oral toxicity study in rats.

4. STUDY IDENTIFICATION: Glaza, S. M. Acute oral toxicity study of SAN 582H 7.5L in rats. (Unpublished study No. HLA 91C03847 conducted by Hazleton Laboratories America, Inc., Madison, WI, and submitted by Sandoz Crop Protection Corp., Des Plaines, IL; dated February 16, 1990.) MRID No. 415965-36.

5. REVIEWED BY:

Patricia A. Turck, M.S.
Principal Reviewer
Dynamac Corporation
Signature: Patricia Turck
Date: January 4, 1991

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation
Signature: Margaret Brower
Date: January 4, 1991

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation
Signature: for Nicolas P. Hajjar
Date: 1-3-91

Deborah McCall
EPA Reviewer, Section III
Toxicology Branch II
(H-7509C)
Signature: Deborah McCall
Date: 1-5-91

James Rowe, Ph.D.
EPA Section Head, Section III
Toxicology Branch II
(H-7509C)
Signature: James N. Rowe
Date: 2/12/91

7. CONCLUSIONS:

CORE Classification: CORE Guideline. This study meets all the requirements set forth under Guideline 81-1 for an acute oral toxicity study in rats.

LD_{50}: 2.0 g/kg, males; 2.8 g/kg, females; and 2.4 g/kg, combined sexes.

Toxicity Category: III.

8. SUMMARY: Groups of five fasted Crl:CD(SD)BR rats/sex/dose (Hazleton Research Products, Inc., Denver, PA), weighing between 214 and 291 g, were administered a single oral dose of 1.0, 2.0, or 5.0 g/kg of the test material (undiluted) and observed 1, 2.5, and 4 hours postdose and daily thereafter for 14 days for clinical signs of toxicity and mortality. Body weights were recorded prior to test material administration, on study day 7, and at study termination. On day 14, surviving animals were killed. All animals were subjected to a gross necropsy, and tissues were discarded.

A total of 1/10, 3/10, and 10/10 rats from the low-, mid-, and high-dose groups, respectively, died during the study; all deaths occurred within the first 3 days after dosing. Clinical signs included hypoactivity, clonic convulsions, tremors, ataxia, excess salivation, diarrhea, red-stained face, and yellow-stained urogenital area. Surviving animals appeared normal by day 3 and gained weight during the study. No findings other than tan, creamy fluid in the stomach were noted at necropsy.

The LD_{50} values were 2.0, 2.8, and 2.4 g/kg for males, females, and combined sexes, respectively.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: The conduct and reporting of the study were adequate; no deficiencies were found. However, the doses were not adjusted to 100% for the active ingredient. The adjusted LD_{50} values are 1.5, 2.1, and 1.83 g/kg for males, females, and sexes combined, respectively.

A signed Quality Assurance Statement, dated February 16, 1990, was provided.

10. CBI APPENDIX: Protocol, CBI pp. 26-32.

APPENDIX A

Protocol
(CBI pp. 26-32)

008285

HAZLETON LABORATORIES AMERICA INC.
3301 KINSMAN BLVD. • P.O. BOX 7548 • MADISON, WI 53707 • (608) 241-4471 • TLX 703858 HAZRAL MDS UD

Sponsor:

Sandoz Crop Protection Corporation
Des Plaines, Illinois

PROTOCOL TP3013

Study Title:

Acute Oral Toxicity Study in Rats
(EPA Guidelines)

Date:

October 20, 1989

Performing Laboratory:

Hazleton Laboratories America, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HLA 91003847

DIMETHENAMID RIN 2014-93

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008285

EPA No.: 68D80056
DYNAMAC No.: 337-B
TASK No.: 3-37B
January 4, 1991

DATA EVALUATION RECORD

SAN 582H

Acute Dermal Toxicity Study in Rabbits

STUDY IDENTIFICATION: Glaza, S. M. Acute dermal toxicity study of SAN 582H 7.5L in rabbits. (Unpublished study No. HLA 91003848 conducted by Hazleton Laboratories America, Inc., Madison, WI, and submitted by Sandoz Crop Protection Corp., Des Plaines, IL; dated February 16, 1990.) MRID No. 415965-37.

APPROVED BY:

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

Signature: Robert J. Weir

Date: 1-3-91

37

008285

1. CHEMICAL: SAN 582H 7.5L.

2. TEST MATERIAL: SAN 582H 7.5L, batch No. 5032-94, contained 76.2% active ingredient and was described as a brown liquid.

3. STUDY/ACTION TYPE: Acute dermal toxicity study in rabbits.

4. STUDY IDENTIFICATION: Glaza, S. M. Acute dermal toxicity study of SAN 582H 7.5L in rabbits. (Unpublished study No. HLA 91003848 conducted by Hazleton Laboratories America, Inc., Madison, WI, and submitted by Sandoz Crop Protection Corp., Des Plaines, IL; dated February 16, 1990.) MRID No. 415965-37.

5. REVIEWED BY:

Patricia Turck, M.S.
Principal Reviewer
Dynamac Corporation
Signature: Patricia Turck
Date: January 4, 1991

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation
Signature: Margaret Brower
Date: January 4, 1991

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation
Signature: RPatrick for H. Hajjar
Date: 1-31-9191

Deborah McCall
EPA Reviewer, Section III
Toxicology Branch II
(H-7509C)
Signature: Deborah McCall
Date: 1-9-91

James Rowe, Ph.D.
EPA Section Head, Section III
Toxicology Branch II
(H-7509C)
Signature: James Rowe
Date: 2/12/91

38

7. CONCLUSIONS:

CORE Classification: CORE Guideline. This study meets all the requirements set forth under EPA Guideline 81-2 for a dermal toxicity study in rabbits.

LD_{50}: >1.5 g (a.i.)/kg for both male and female rabbits.

Toxicity Category: III. No systemic toxicity was observed; however, severe dermal irritation was noted.

8. SUMMARY: The undiluted test material, at a dose of 2 g/kg, was applied to the intact skin of five New Zealand White rabbits [Hra:(NZW)SPF, Hazleton Research Products, Denver, PA] of each sex. Body weights ranged from 2.09 to 2.48 kg at study initiation. Approximately 24 hours prior to study initiation, the backs were shaved to expose approximately 20% of body surface. The test sites were covered with a gauze patch (10 cm), which was held in place with paper tape and covered with plastic wrap. The rabbits were restrained with collars for a 24-hour period. After the bandages were removed, the remaining test material was washed off with warm water, and test sites were dried. Animals were observed 1, 2.5, and 4 hours postdose, and daily, thereafter, for 14 days for clinical signs of toxicity. Dermal irritation was scored using the method of Draize 30 minutes after removal of bandages and on study days 3, 7, 10, and 14. Body weights were recorded prior to study initiation, on study day 7, and prior to study termination. Survivors were killed on study day 14, and all animals were subjected to a gross necropsy.

No deaths were observed and all animals appeared normal throughout the study period. Severe dermal irritation, consisting of slight to severe erythema and edema, atonia, coriaceousness and fissuring, desquamation, subcutaneous hemorrhaging, blanching, exfoliation, and eschar formation, was noted. Animals consistently gained weight during the study, and necropsy revealed no abnormal findings. Based on the results, the LD_{50} value was >2.0 g/kg for both male and female rabbits.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: The conduct and reporting of this study were adequate; no deficiencies were found. An LD_{50} value of >2 g/kg was identified; this is the maximum dose required as the limit test by EPA Guidelines (1984). However, the dose was not adjusted for % active ingredient, and therefore, the actual LD_{50} was >1.5 g/kg. It does, however, cause severe dermal irritation.

A signed Quality Assurance Statement, dated February 16, 1990, was provided.

10. CBI APPENDIX: Protocol, CBI pp. 18-25.

40

APPENDIX

Protocol

(CBI pp. 18-25)

008285

HAZLETON LABORATORIES AMERICA, INC.
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703856 HAZRAL MDS UD

Sponsor:

Sandoz Crop Protection Corporation
Des Plaines, Illinois

PROTOCOL TP3016

Study Title:

Acute Dermal Toxicity Study in Rabbits
(EPA Guidelines)

Date:

October 20, 1989

Performing Laboratory:

Hazleton Laboratories America, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HLA 91003848

0042

DIMETHENAMID RIN 2014-93

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Pages 43 through 49 are not included.

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____ 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.

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008285

EPA No.: 68D80056
DYNAMAC No.: 337-C
TASK No.: 3-37C
January 4, 1991

DATA EVALUATION RECORD

SAN 582H

Acute Inhalation Toxicity Study in Rats (4-Hour Exposure)

STUDY IDENTIFICATION: Jackson, G. C. and C. J. Hardy. SAN 582H 7.5L. Acute inhalation toxicity study in rats, 4-hour exposure. (Unpublished study No. SNC84/90352, conducted by Huntingdon Research Center Ltd., Cambridgeshire, England, and submitted by Sandoz Crop Protection Corp., Des Plaines, IL; dated April 6, 1990.) MRID 415965-38.

APPROVED BY:

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

Signature: Robert J. Weir

Date: 3/7/95

0000

008285

1. CHEMICAL: SAN 582H.

2. TEST MATERIAL: SAN 582H 7.5L, batch No. 5032-94, was 76.2% pure and described as a brown liquid.

3. STUDY/ACTION TYPE: Acute inhalation toxicity study in rats (4-hour exposure).

4. STUDY IDENTIFICATION: Jackson, G. C. and C. J. Hardy. SAN 582H 7.5L. Acute inhalation toxicity study in rats, 4-hour exposure. (Unpublished study No. SNC84/90352, conducted by Huntingdon Research Center Ltd., Cambridgeshire, England, and submitted by Sandoz Crop Protection Corp., Des Plaines, IL; dated April 6, 1990.) MRID 415965-38.

5. REVIEWED BY:

Patricia Turck, M.S.
Principal Reviewer
Dynamac Corporation
Signature: Patricia Turck
Date: January 4, 1991

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation
Signature: Margaret E. Brower
Date: January 4, 1991

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation
Signature:
Date: 1/3/91

Deborah McCall
EPA Reviewer, Section III
Toxicology Branch II
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Signature: Deborah McCall
Date: 1-5-91

James Rowe, Ph.D.
EPA Section Head, Section III
Toxicology Branch II
(H-7509C)
Signature: James Rowe
Date: 2/12/91

7. CONCLUSIONS:

Core Classification: CORE Minimum. This study meets the minimum requirements set forth under Guideline 81-3 for an acute inhalation toxicity study in rats.

LC_{50}: >3.39 mg/L for SAN 582H 7.5L
>2.58 mg/L for SAN 582H (adjusted for purity).

Toxicity Category: III.

8. SUMMARY: A group of five Sprague-Dawley rats/sex (Charles River (UK) Limited, Kent, England), weighing between 206 and 248 g, were exposed via whole-body inhalation to an aerosol of the test material for 4 hours. An additional group of five rats/sex was exposed to air only and served as controls.

The test atmosphere was generated by pumping the test material through an atomizer using a syringe pump located at the bottom of a 120-L perspex inhalation chamber. The flow rate for the test material was approximately 0.6 mL/min, the maximum attainable rate. The carrier gas was dried, filtered, compressed air, and airflow was maintained at approximately 25 L/min. Aerosol concentration was analyzed gravimetrically five times during the 4-h exposure, and particle size was determined twice using a May multistage liquid impinger (methanol, trapping agent).

Animals were observed continuously during the exposure and twice daily, thereafter, for 14 days for mortality and clinical signs of toxicity. Body weight and food and water consumption were recorded daily. Fourteen days after exposure, surviving animals were killed and subjected to a gross necropsy. Lungs were weighed and infused, and liver, kidneys, and lungs were preserved for possible future histopathological examination.

The mean aerosol concentration was 3.39 mg/L SAN 582H 7.5L or 2.58 mg/L SAN 582H (adjusted for purity). This was the maximum attainable concentration under the conditions of this study. Approximately 91% of particles were <5.5 µm in diameter; the mass median aerodynamic diameter was 1.0 µm, and 49% (by weight) of the generated particles were ≤1.0 µm. No deaths occurred during the study. Clinical signs during the exposure included partial closing of eyes, salivation, reduced respiratory rate, and abnormal body posture. Signs noted after exposure included wet, matted fur; brown staining around the snout and jaws; ocular discharge; lethargy; abnormal respiration; and ataxia. By days 8 and 13, all males and females, respectively, appeared normal. Body weight and food consumption were reduced in test animals during the first 3

days following exposure but returned to control levels thereafter, except in one male and one female. Water consumption was variable or reduced in females during the observation period and reduced for males on day 1. No abnormal findings were noted at necropsy. Relative lung weight (to body weight) was similar between control and test groups. Based on the results, the 4-h LC_{50} >3.39 mg/L.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: The conduct and reporting of this study were adequate, except that a protocol was not provided. The exposure concentration was adjusted for purity of the test material. The LC_{50} (4 h) for pure test material is >2.58 mg/L. This was the maximum attainable aerosol concentration because the test material was reportedly "cohesive." Particle size was acceptable under study conditions; approximately 70% were <2.0 μm. Owing to the sticky nature of the test material, a slight increase in the percentage of particles >5.5 μm was observed after 3 hours.

   A signed Quality Assurance Statement, dated April 6, 1990 was provided.

10. CBI APPENDIX: Not applicable.

008285

EPA No.: 68D80056
DYNAMAC No.: 337-D
TASK No.: 3-37D
January 4, 1991

DATA EVALUATION RECORD

SAN 582H

Primary Eye Irritation Study in Rabbits

STUDY IDENTIFICATION: Glaza, S. M. Primary eye irritation study of SAN 582H 7.5L in rabbits. (Unpublished study No. HLA 91003350 conducted by Hazleton Laboratories America, Inc., Madison, WI, and submitted by Sandoz Crop Protection Corp., Des Plaines, IL; dated February 16, 1990.) MRID No. 415965-39.

APPROVED BY:

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

Signature: Robert J. Weir

Date: 1-3-91

0051

1. CHEMICAL: SAN 582H 7.5L.

2. TEST MATERIAL: SAN 582H 7.5L, batch No. 5032-94, contained 76.2% active ingredient and was described as a brown liquid. The pH was 3.9.

3. STUDY ACTION TYPE: Primary eye irritation study in rabbits.

4. STUDY IDENTIFICATION: Glaza, S. M. Primary eye irritation study of SAN 582H 7.5L in rabbits. (Unpublished study No. HLA 91003850 conducted by Hazleton Laboratories America, Inc., Madison, WI, and submitted by Sandoz Crop Protection Corp., Des Plaines, IL; dated February 16, 1990). MRID No. 415965-39.

5. REVIEWED BY:

Patricia A. Turck, M.S.
Principal Reviewer
Dynamac Corporation
Signature: Patricia Turck
Date: January 4, 1991

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation
Signature: Margaret Brower
Date: January 4, 1991

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation
Signature: for Nicolas P. Hajjar
Date: 1/3/91

Deboran McCall
EPA Reviewer, Section III
Toxicology Branch II
(H-7509C)
Signature: Deborah McCall
Date: 1-9-91

James Rowe, Ph.D.
EPA Section Head, Section III
Toxicology Branch II
(H-7509C)
Signature: James Rowe
Date: 2/12/91

7. CONCLUSIONS:

CORE Classification: CORE Guideline. This study meets all the requirements set forth under Guideline 81-4 for a primary eye irritation study in rabbits.

Primary Eye Irritation Index: Not reported.

Toxicity Category: II. Corneal involvement and irritation cleared within 21 days after dosing.

8. SUMMARY: Into one eye of each of six New Zealand White rabbits [HRA:(NZW)SPF, Hazleton Research Products, Inc., Denver, PA], 0.1 mL of undiluted test material (pH 3.9) was instilled; the eye was held closed for 1 second. No washing was performed. Prior to dosing, the eyes were screened with sodium fluorescein to exclude rabbits with ocular injury or irritation. Irritation was scored 1, 24, 48, 72, and 96 hours, and 7 and 21 days postdose, using the method of Draize. Sodium fluorescein was used to aid in the determination of possible corneal damage. Body weights were recorded prior to study initiation, on day 7, and at study termination.

Slight to severe conjunctival irritation, slight corneal opacity, and iritis were observed up to 7 days after instillation. By day 21, all eyes appeared normal.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: The conduct and reporting of the study were adequate; no deficiencies were found. The test material was very irritating to the eye, but all corneal opacity and irritation had cleared by day 21.

A signed Quality Assurance Statement, dated February 16, 1990, was provided.

10. CBI APPENDIX: Protocol, CBI pp. 22-30.

APPENDIX A

Protocol
(CBI pp. 22-30)

HAZLETON LABORATORIES AMERICA INC
3301 KINSMAN BLVD. • P.O. BOX 7548 • MADISON, WI 53707 • (608) 241-4471 • TLX 703856 HAZRAL MDS UD

Sponsor:

Sandoz Crop Protection Corporation
Des Plaines, Illinois

PROTOCOL TP3015

Study Title:

Primary Eye Irritation Study in Rabbits
(EPA Guidelines)

Date:

October 20, 1989

Performing Laboratory:

Hazleton Laboratories America, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HLA 91003850

DIMETHENAMID RIN 2014-93

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EPA No.: 68D80056
DYNAMAC No.: 337-E
TASK No.: 3-37E
January 4, 1991

DATA EVALUATION RECORD

SAN 582H

Primary Dermal Irritation Study in Rabbits

STUDY IDENTIFICATION: Glaza, S. M. Primary dermal irritation study of SAN 582H 7.5L in rabbits. (Unpublished study No. HLA 91003849 conducted by Hazleton Laboratories America, Inc., Madison, WI, and submitted by Sandoz Crop Protection Corp., Des Plaines, IL; dated February 16, 1990.) MRID No. 415965-40.

APPROVED BY:

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

Signature: Robert J. Weir

Date: 1-3-91

0067

008285

1. CHEMICAL: SAN 582H 7.5L.

2. TEST MATERIAL: SAN 582H 7.5L, batch No. 5032-94, contained 76.2% active ingredient and was described as a brown liquid.

3. STUDY/ACTION TYPE: Primary dermal irritation study in rabbits.

4. STUDY IDENTIFICATION: Glaza, S. M. Primary dermal irritation study of SAN 582H 7.5L in rabbits. (Unpublished study No. HLA 91003849 conducted by Hazleton Laboratories America, Inc., Madison, WI, and submitted by Sandoz Crop Protection Corp., Des Plaines, IL; dated February 16, 1990.) MRID No. 415965-40.

5. REVIEWED BY:

Patricia A. Turck, M.S.
Principal Reviewer
Dynamac Corporation
Signature: Patricia Turck
Date: January 4, 1991

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation
Signature: Margaret Brower
Date: January 4, 1991

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation
Signature: [illegible]
Date: 1-3-91

Deborah McCall
EPA Reviewer, Section III
Toxicology Branch II
(H-7509C)
Signature: Deborah McCall
Date: 1-9-91

James Rowe, Ph.D.
EPA Section Head, Section III
Toxicology Branch II
(H-7509C)
Signature: James Rowe
Date: 2/12/91

7. CONCLUSIONS:

CORE Classification: CORE Guideline. This study meets all the requirements set forth under Guideline 81-5 for a primary dermal irritation study in rabbits.

Primary Dermal Irritation Index: 0.6, slightly irritating.

Toxicity Category: IV. Mild irritation at 72 hours.

8. SUMMARY: Twenty-four hours before study initiation, the backs and flanks of six New Zealand White rabbits [HRA;(NZW)SPF, Hazleton Research Products, Denver, PA], weighing 2.20 to 2.5 kg, were shaved. To the intact skin, 0.5 mL of the undiluted test material was applied. The test sites were covered with 2.5- x 2.5-cm gauze patches held in place with paper tape and protected with plastic wrap for 4 hours. Rabbits were restrained during the exposure period with collars. After the exposure period, the patches were removed, and remaining test material was washed off with warm water. Irritation was scored using the method of Draize 30 minutes, and 24, 48, 72, and 96 hours postexposure.

Slight to well-defined erythema was noted up to 72 hours and very slight edema was noted up to 48 hours postexposure. No irritation was observed 96 hours after exposure. The mean Primary Dermal Irritation Score was 0.6, slightly irritating.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: The conduct and reporting of this study were adequate. The test material was slightly irritating to the intact skin.

A signed Quality Assurance Statement, dated February 16, 1990, was provided.

10. CBI APPENDIX: Protocol, CBI pp. 12-19.

APPENDIX A

Protocol
(CBI pp. 12-19)

008285

HAZLETON LABORATORIES AMERICA INC.
3301 KINSMAN BLVD. • P.O. BOX 7548 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

Sponsor:

Sandoz Crop Protection Corporation
Des Plaines, Illinois

PROTOCOL TP3014

Study Title:

Primary Dermal Irritation Study in Rabbits
(EPA Guidelines)

Date:

October 20, 1989

Performing Laboratory:

Hazleton Laboratories America, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HLA 91003849

0071

DIMETHENAMID RIN 2014-93

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_____ The product confidential statement of formula.

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008285

EPA No.: 68D80056
DYNAMAC No.: 337-F
TASK No.: 3-37F
January 4, 1991

DATA EVALUATION RECORD

SAN 582H

Dermal Sensitization Study in Guinea Pigs
(Buehler Test)

STUDY IDENTIFICATION: Glaza, S. M. Dermal sensitization study of SAN 582H 7.5L in guinea pigs -- closed patch technique. (Unpublished study No. HLA 91003851 conducted by Hazleton Laboratories America, Inc., Madison, WI, and submitted by Sandoz Crop Protection Corp., Des Plaines, IL; dated February 16, 1990.) MRID No. 415965-41.

APPROVED BY:

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

Signature: Robert J. Weir

Date: 1-4-91

0079

1. CHEMICAL: SAN 582H 7.5L.

2. TEST MATERIAL: SAN 582H 7.5L, batch No. 5032-94, contained 76.2% active ingredient and was described as a brown liquid.

3. STUDY/ACTION TYPE: Dermal sensitization study in guinea pigs.

4. STUDY IDENTIFICATION: Glaza, S. M. Dermal sensitization study of SAN 582H 7.5L in guinea pigs -- closed patch technique. (Unpublished study No. HLA 91003851 conducted by Hazleton Laboratories America, Inc., Madison, WI, and submitted by Sandoz Crop Protection Corp., Des Plaines, IL; dated February 16, 1990.) MRID No. 415965-41.

5. REVIEWED BY:

Patricia A. Turck, M.S.
Principal Reviewer
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Signature: Patricia Turck
Date: January 4, 1991

Margaret E. Brower, Ph.D.
Independent Reviewer
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Date: January 4, 1991

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation
Signature: William L McLellan for
Date: Jan 4 1991

Deborah McCall
EPA Reviewer, Section III
Toxicology Branch II
(H-7509C)
Signature: Deborah McCall
Date: 1-9-91

James Rowe, Ph.D.
EPA Section Head, Section III
Toxicology Branch II
(H-7509C)
Signature: James Rowe
Date: 2/12/91

7. CONCLUSIONS:

CORE Classification: CORE Guideline. This study meets all the requirements set forth under Guideline 81-6 for a dermal sensitization study in guinea pigs.

Skin Sensitization Potential: SAN 582H 7.5L is a dermal sensitizer, eliciting slight to moderate dermal reactions in 10 guinea pigs 24 hours after dermal challenge.

8. SUMMARY: Prior to initiation of the definitive study, two range-finding studies were conducted to choose a dose level for the induction and challenge phases. The results of the first range-finding study were not used for dose selection because of conflicting findings. In a subsequent range-finding study, a total of four HRA:(DH)SPF guinea pigs/sex (Hazleton Research Products, Inc., Denver, PA) received dermal applications of 25, 50, 75, or 100% test material (w/v) in deionized water. Based on the results, undiluted (100%) test material was used. For the induction phase, doses of 0.4 mL of undiluted test material (100%) were applied to 25-mm diameter adhesive pads and placed on the shaved left flanks of five guinea pigs/sex. The pads were covered and held in place with tape for 6 hours. The bandages were then removed, and remaining test material was wiped off with damp paper towels. A group of two guinea pigs/sex received dermal applications of 0.3% (w/v) 2,4-dinitrochlorobenzene (DNCB) in 80% ethanol/deionized water and served as positive control animals. The test and positive control groups were administered one dermal application/week for 3 weeks.

During the challenge phase (2 weeks after the last induction application), the undiluted test material was applied to the right flank of each test animal. In addition, five previously untreated guinea pigs/sex received a dermal application of undiluted test material (naive controls). Positive control animals received 0.4 mL 0.1% DNCB (w/v) in acetone. Dermal reactions were scored using the Buehler method 24 hours after the challenge application. Approximately 3 hours before scoring, the hair on test sites was removed using Neet. Animals were observed daily for clinical signs, and body weights were recorded on study days 1, 8, 15, 22, and 29, and at study termination.

Body weight gains were similar for control and test animals during the study. Slight to moderate irritation (erythema) with subcutaneous hemorrhage was observed in test animals during the induction phase. Upon challenge at previously untreated sites on the test animals, slight to strong dermal reactions (erythema) were observed. Naive controls did not exhibit any reaction; no irritation was observed. Positive

control animals exhibited moderate to strong dermal reactions with subcutaneous hemorrhaging during both induction and challenge phases. Based on the results of the challenge phase, the undiluted test material is a dermal sensitizer in guinea pigs.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: The conduct and reporting of the study were adequate; no deficiencies were found. SAN 582H 7.5L is a dermal sensitizer, eliciting slight to moderate dermal reactions upon challenge.

   A signed Quality Assurance Statement, dated February 16 1990, was provided.

10. CBI APPENDIX: Protocol, CBI pp. 22-30.

APPENDIX

Protocol
(CBI pp. 22-30)

008285

HAZLETON LABORATORIES AMERICA INC.
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

Sponsor:

Sandoz Crop Protection Corporation
Des Plaines, Illinois

PROTOCOL TP2008

Study Title:

Dermal Sensitization Study in Guinea Pigs -
Closed Patch Technique
(EPA Guidelines)

Date:

October 20, 1989

Performing Laboratory:

Hazleton Laboratories America, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HLA 91003851

DIMETHENAMID RIN 2014-93

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_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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__V__ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

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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.

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DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

008285

EPA No.: 68D80056
DYNAMAC No.: 337-K
TASK No.: 3-37K
February 5, 1991

DATA EVALUATION RECORD

SAN 582 H

Subchronic Feeding Study in Rats

APPROVED BY:

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

Signature: William L. McLellan for

Date: Feb. 5, 1991

0093

008285

EPA No.: 68D80056
DYNAMAC No.: 337-K
TASK No.: 3-37K
February 5, 1991

DATA EVALUATION RECORD

SAN 582 H

Subchronic Feeding Study in Rats

REVIEWED BY:

Patricia A. Turck, M.S.
Principal Reviewer
Dynamac Corporation
Signature: Patricia Turck
Date: February 5, 1991

Margaret E. Brower, Ph.D.
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Date: February 5, 1991

APPROVED BY:

Nicolas P. Hajjar, Ph.D.
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Deborah McCall
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Date: 2-11-91

James Rowe, Ph.D.
EPA Section Head
Section III
Toxicology Branch II
(H-7509C)
Signature: James Rowe
Date: 2/12/91

008285

DATA EVALUATION RECORD

GUIDELINE §82-1

STUDY TYPE: Subchronic feeding study in rats.

MRID NUMBER: 416159-01.

TEST MATERIAL: SAN 582 H.

SYNONYM: N/A.

STUDY NUMBER: SDI 327/87313.

SPONSOR: Sandoz Crop Protection Corporation, Des Plaines, IL.

TESTING FACILITY: Huntingdon Research Centre Ltd , Cambridgeshire, England.

TITLE OF REPORT: Toxicity to Rats by Repeated Dietary Administration for 13 Weeks Followed by a 4-Week Withdrawal Period.

AUTHORS: Ruckman, S. A.; Anstey, M. C.; Heywood, R.; Crook, D.; Gopinath, C.; Gibson, N. A.; Anderson, A.

REPORT ISSUED: October 16, 1986.

CONCLUSIONS:

In a 13-week subchronic toxicity study in which groups of Sprague-Dawley rats were fed diets containing SAN 582 H at concentrations of 0, 50, 150, 500, 1500, or 3000 ppm (approximately 0, 3.5, 10.0, 33.5, 98, or 204 mg/kg/day for males and 0, 3.9, 11.8, 40.1, 119, or 238 mg/kg/day for females, respectively), compound-related reductions in body weight, increases in relative liver weight, centrilobular hepatocytic enlargement, and increased protein and cholesterol levels were observed at 1500 ppm or higher. Based on these changes, which are indicative of hepatic toxicity, the NOEL and LOEL were 500 and 1500 ppm, respectively.

Classification: CORE Minimum. This study meets the minimum requirements set forth under Guideline 82-1 for a subchronic toxicity study in rats.

A. MATERIALS:

1. Test Compound: SAN 582 H; description: brown oil; batch No.: 8605; purity: approximately 91.5%.

2. Test Animals: Species: rat; strain: Sprague-Dawley; age: approximately 34 days; weight: males--223 to 284 g, females--142 to 195 g at study initiation; source: Charles River, USA.

B. STUDY DESIGN:

1. Animal Assignment: After an acclimation period of 6 days, animals were randomly assigned to the following test groups according to body weight (all rats were within ± 8% of the mean):

Test group	Dietary Concentration (ppm)	Main Study (13 Weeks)		Recovery (4 Weeks)	
		Males	Females	Males	Females
1 Control	0	10	10	10	10
2 Low (LDT)	50	10	10		
3 Mid A (MDT)	150	10	10		
4 Mid B (MDT)	500	10	10		
5 Mid C (MDT)	1500	10	10		
6 High (HDT)	3000	10	10	10	10

Besides the 10 rats/sex/group assigned for the main study, 10 additional animals/sex were assigned to the control and 3000-ppm groups. These animals were observed for an additional 4 weeks after the 13-week test period to determine the extent of recovery. Prior to study initiation, 10 animals/sex were used to establish baseline values for hematology and clinical chemistry parameters. After being assigned to test groups, animals were acclimated for an additional 14 days prior to initiation of treatment. Rats showing abnormal clinical signs were replaced with extra animals before study initiation. Rats were housed five/cage during the acclimation and study periods. Each rat was uniquely identified using an eartag and leg tattoo. Room temperature and relative humidity were monitored daily and maintained at approximately 21°C and 55%, respectively. A 12-hour light/dark cycle was provided.

2. Diet Preparation: Test diets were prepared weekly and administered to rats for 13 weeks. The recovery groups were maintained on control diet for an additional 4 weeks. A premix, prepared by adding the test material dissolved in acetone (removed by evaporation) to SDS Rat and Mouse No. 1 modified maintenance diet, was diluted with control diet to achieve the desired concentrations. The test diets were then mixed for a minimum of 7 minutes using a double cone blender. Control animals received diet prepared by adding acetone, which was then evaporated as it had been for the test diets. Analyses were conducted on diets prepared during weeks 1 and 13. Homogeneity and stability (at 30 and 3000 ppm) were determined prior to study initiation.

Results: Chemical analysis revealed that test diets were within ± 9% of nominal concentrations and ranged from 94.6 to 106.4%. Homogeneity of the actual test diets was not demonstrated; the study authors presented data from an analysis conducted prior to study initiation, which demonstrated that the preparation method produced homogeneous test diets. Aliquots from the top, middle, and bottom were within 4% of each other and ranged from 102 to 113.3% of the nominal concentration of 30 ppm and from 92.7 to 101.7% of the nominal concentration of 3000 ppm. The test diets were found to be stable for at least 11 days under animal room conditions (at room temperature, exposed to the atmosphere).

3. Food and Water Consumption: Animals were fed diets and water (from water bottles) ad libitum during the study.

4. Statistics: The following procedures were utilized in analyzing body weights, food consumption, water consumption (food and water consumption were calculated on a per cage basis), organ weight, and clinical pathology data. If data consisted mainly of one value, i.e., the relative frequency of the mode was >75%, the proportion of animals having values different from the mode was analyzed using Fisher's and Mantel's tests. Heterogeneity of data was determined using Bartlett's test. If data were heterogeneous, logarithmic transformation was attempted. If unsuccessful, Kruskal-Wallis analysis of ranks followed by a non-parametric equivalent of the T-test and Williams test was used. Homogeneous data were analyzed using one-way ANOVA, followed by Student's T-test and Williams test; and final body weight was used as a covariate in the analysis of organ weight data.

5. Quality Assurance: Signed Quality Statements, dated October 8 and 23, 1987, were provided.

C. METHODS AND RESULTS:

1. Observations: Animals were observed for mortality and moribundity twice daily throughout the study period. In addition, clinical and behavioral signs of toxicity were recorded daily except on weekends for the first 4 weeks of the study and once weekly, thereafter.

Results: No deaths occurred, and no abnormal clinical or behavioral signs of toxicity were observed during the study that could be attributed to the test material. The following signs were observed with equal frequency among animals from the control and test groups: transient red or brown perinasal or periocular staining, which was generally associated with malocclusion of incisors, alopecia, swollen muzzle, ulcerations on the face or under the tail, red- or brown-stained and swollen pinnae, and transient reduction in grooming behavior. Other signs included hypersensitivity, which was observed in two control females and one 150-ppm female, and transient piloerection and respiratory distress in one 3000-ppm female. In addition, one 500-ppm female exhibited dark extremities, respiratory distress, hyperactive behavior, eyes with dry surface, twitching, subdued appearance, lethargy, and unsteady gait for 1 day only, at the end of study week 12.

2. Body Weight: Body weight was recorded weekly during the study.

Results: Body weight and body weight gain (days 0-91) data are summarized in Tables 1 and 2. Significant reductions ($p < 0.05$ to 0.01) in body weight gain were observed after 13 weeks in males administered 1500 or 3000 ppm and females in the 3000-ppm group. Statistical analysis by the reviewers using Dunnett's test and ANOVA revealed significant ($p < 0.01$) reductions in the body weight of high-dose animals at weeks 7 and 13. Furthermore, statistical analyses by the reviewers revealed significant reductions in body weight gain in high-dose animals ($p < 0.05$-0.01) during weeks 0 to 7 and 7 to 13, and in mid-dose males ($p < 0.05$) receiving 1500 ppm during weeks 7 to 13. Slight, but nonsignificant reductions in body weight gains were also observed in females ingesting 1500 ppm (13 to 21%) and males fed 500 ppm (4 to 13%) during the 13-week study.

During the 4-week recovery period, a compensatory, significant increase ($p < 0.05$) in body weight gain was observed in the 3000-ppm males, indicating that effects on

TABLE 1. Mean Body Weights at Selected Intervals and Total Body Weight Gain in 13-Week Dietary Toxicity Study of SAN 582 H in Rats[a]

Dietary Concentration (ppm)	Mean Body Weight (g ± S.D.) at Study Week: 0	7	13	17[c]
		Males		
0	242 ± 11.9	452 ± 27.8	500 ± 36.0	535 ± 34.5
50	238 ± 10.1	455 ± 39.3	506 ± 50.5	--[b]
150	247 ± 10.6	459 ± 48.1	498 ± 60.5	--
500	242 ± 7.4	444 ± 26.2	486 ± 32.3	--
1500	239 ± 15.7	421 ± 45.5	454 ± 52.5*	--
3000	243 ± 14.8	409 ± 39.2**	443 ± 44.5**	512 ± 48.4
		Females		
0	166 ± 7.9	248 ± 15.9	266 ± 17.7	269 ± 13.2
50	166 ± 7.0	245 ± 15.7	261 ± 18.8	--
150	164 ± 6.9	250 ± 13.9	261 ± 11.5	--
500	168 ± 12.7	247 ± 22.3	262 ± 26.0	--
1500	167 ± 9.0	239 ± 14.8	254 ± 19.3	--
3000	164 ± 9.1	228 ± 10.2**	240 ± 13.2**	254 ± 18.0*

[a]Statistical analysis performed by reviewers using Dunnett's test and ANOVA.

[b]Indicates no data available. Animals from 50-, 150-, 500-, and 1500-ppm groups were killed after week 13.

[c]Termination after 4-week recovery period.

*Significantly different from controls ($p < 0.05$).

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

TABLE 2. Mean Body Weight Gains in 13-Week Dietary Toxicity Study of SAN 582 H in Rats[a]

Dietary Concentration (ppm)	Mean Body Weight Gain (g ± S.D.) at Study Week: 0-7	7-13	0-13
		Males	
0	211 ± 20.2	48 ± 14.0	259 ± 29.5
50	217 ± 32.5	51 ± 16.4	268 ± 43.6
150	213 ± 38.9	39 ± 15.8	252 ± 51.2
500	202 ± 23.3	42 ± 9.7	244 ± 29.7
1500	182 ± 32.2	33 ± 16.4*	215 ± 39.6*
3000	165 ± 29.5**	35 ± 14.3*	200 ± 36.3**
		Females	
0	82 ± 11.6	18 ± 7.6	100 ± 14.7
50	79 ± 12.4	16 ± 6.3	94 ± 16.4
150	85 ± 11.5	11 ± 4.7	96 ± 12.1
500	79 ± 12.6	15 ± 8.7	94 ± 17.6
1500	73 ± 7.2	15 ± 8.5	87 ± 13.8
3000	64 ± 7.4**	12 ± 7.7*	76 ± 11.1**

[a]Statistial analysis conducted by the reviewers using Dunnett's test and ANOVA.

*Significantly different from controls ($p < 0.05$).

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

body weight were reversible after termination of test material administration. Increases were also observed in high-dose females, but the week 17 body weights remained significantly ($p < 0.05$) below those of control females.

3. Food Consumption and Compound Intake: Total food consumption for each cage was measured and recorded weekly. Food consumption/rat was calculated based on total food consumption/cage and the number of rats/cage. Food efficiency and test material intake were calculated based on mean food consumption and mean body weight data.

   Results: Food consumption for rats ingesting 1500 or 3000 ppm was slightly reduced during the study. Total food consumption (g/rat for 13 weeks) was significantly reduced ($p < 0.05$) in females at the high dose. The study authors reported "marginal" compound-related reductions in food consumption at 1500 and 3000 ppm. The food consumption values presented in the report were difficult to interpret because summary and individual data were reported in units of g/rat/day; however, the animals were group-housed and statistics were reportedly performed on cage values (See Section E for further discussion).

   Food utilization was decreased (nonsignificant) in high-dose male and female rats and females ingesting 1500 ppm during the treatment period. Test material intake for males and females was 3.5-3.9, 10.0-11.8, 33.5-40.1, 98-119, and 204-238 mg/kg/day for the 50-, 150-, 500-, 1500-, and 3000-ppm groups, respectively.

   During the recovery period, food consumption and utilization were similar between control and high-dose animals.

4. Water Consumption: Water consumption was measured by weight daily during week 12.

   Results: Water intake was slightly lower in high-dose females when compared with controls during week 12. The study authors attributed this to a corresponding reduction in food consumption in high-dose females during this week. However, the reviewers regard the reduction in water consumption as a possible compound-related effect.

5. Ophthalmological Examinations: Ophthalmological examinations using a Keeler indirect ophthalmoscope were performed prior to study initiation and during week 12 of the study on the eyes of all control and high-dose animals. Pupils were dilated with Mydriacyl (Alcon Laboratories, Inc., Fort Worth, TX) prior to examination.

Results: No compound-related abnormalities were noted.

6. Hematology and Clinical Chemistry: Baseline hematological and clinical chemistry parameters were obtained prior to study initiation using 10 rats/sex (not fasted). Blood samples were collected during week 13 of the study from the orbital sinus of all animals. Rats were fasted overnight and anesthetized with ether prior to blood collection. Blood was also collected from recovery animals in a similar manner during week 17. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
Reticulocyte count (RETIC)
X Cell morphology
X Leukocyte differential count
Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Thrombotest:prothrombin time

Results: Slight but significant reductions in MCHC ($p < 0.01$) and platelet count ($p < 0.05$) were observed in males fed 500 ppm or higher at week 13. Slight but significant increases ($p < 0.05$) in packed cell volume (hematocrit) and MCV were observed in high-dose males. In females, prothrombin time was significantly reduced ($p < 0.01$) in all test groups when compared with controls, and the platelet count was significantly increased ($p < 0.05$-0.01) at 150 ppm or higher. Since a dose-relationship was not evident and the values were within expected ranges for this strain and age of rat, the changes were not considered to be compound related. After the 4-week recovery period, a slight but significant reduction ($p < 0.05$) in MCV was observed in high-dose females. Other values were comparable between control and high-dose animals.

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*Recommended by Subdivision F (November 1984) Guidelines for subchronic oral toxicity studies.

b. Clinical Chemistry:

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

Results: Selected clinical chemistry data collected at week 13 are summarized in Tables 3 and 4. Significant, compound-related increases ($p < 0.01$) in total protein were observed in both males and females fed 1500 or 3000 ppm. Globulins were significantly increased in high-dose males ($p < 0.01$) and females ($p < 0.01$) receiving 150 ppm or higher, and albumin was significantly higher ($p < 0.01$) in males fed 150 ppm or higher. Slight but significant ($p < 0.05$) changes in electrolyte levels occurred in dosed males and females.

The study authors considered the marginal but significant increases in globulin in females and albumin in males at 150 and 500 ppm to be of little toxicological significance because an accompanying increase in total protein was not observed at these levels. The decreases in SGOT and SGPT in animals fed 3000 ppm were of no toxicological significance. In addition, changes in these parameters were slight and were within the range of those of historical controls.[1] All other changes in clinical

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[1]Hazleton Laboratories. 1984. Representative Historical Control Data. Clinical Chemistry Reference Ranges in Sprague-Dawley Rats.

†Recommended by Subdivision F (November 1984) Guidelines for subchronic oral toxicity studies.

TABLE 3. Selected Terminal Clinical Chemistry Data (Mean ± S.D.) in a 13-Week Dietary Toxicity Study of SAN 582 H in Male Rats

Parameter	Dietary Concentration (ppm): 0	50	150	500	1500	3000
Total protein (g/dL)	6.7 ± 0.21	7.0 ± 0.22	6.8 ± 0.22	6.8 ± 0.19	7.1 ± 0.26**	7.4 ± 0.25**
Albumin (g/dL)	3.8 ± 0.14	3.9 ± 0.21	4.0 ± 0.16**	4.0 ± 0.14**	4.2 ± 0.13**	4.2 ± 0.12**
Globulin (g/dL)	2.9 ± 0.15	3.1 ± 0.18	2.8 ± 0.18	2.8 ± 0.13	3.0 ± 0.18	3.2 ± 0.24**
Cholesterol (g/dL)	73.4 ± 13.82	67.9 ± 15.60	75.9 ± 22.0	76.7 ± 18.02	87.2 ± 23.33	103.9 ± 22.95**
SGPT (mU/mL)	23 ± 6.7	21 ± 3.3	19 ± 2.9*	17 ± 4.2*	19 ± 4.4*	19 ± 3.2**
SGOT (mU/mL)	61 ± 10.6	53 ± 5.2	49 ± 3.9*	51 ± 12.2*	46 ± 3.4**	42 ± 3.4**
Urea nitrogen (mg/dL)	14 ± 1.9	14 ± 2.9	14 ± 1.6	14 ± 1.0	16 ± 2.8*	17 ± 2.0**
Creatinine (mg/dL)	0.6 ± 0.08	0.7 ± 0.07	0.6 ± 0.08	0.6 ± 0.08	0.6 ± 0.09	0.6 ± 0.09
GGT (mU/mL)	2 ± 0.8	1 ± 0.4	2 ± 1.0	1 ± 0.8	2 ± 1.2	6 ± 1.3**
Bilirubin (mg/dL)	0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.02**

*Significantly different from controls ($p < 0.05$).

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

TABLE 4. Selected Terminal Clinical Chemistry Data (Mean ± S.D.) in a 13-Week Dietary Toxicity Study of SAN 582 H in Female Rats

Parameter	Dietary Concentration (ppm): 0	50	150	500	1500	3000
Total protein (g/dL)	6.9 ± 0.45	6.9 ± 0.24	7.1 ± 0.48	7.2 ± 0.23	7.4 ± 0.20**	7.3 ± 0.21**
Albumin (g/dL)	4.3 ± 0.40	4.2 ± 0.16	4.3 ± 0.38	4.3 ± 0.22	4.4 ± 0.21	4.4 ± 0.18
Globulin (g/dL)	2.6 ± 0.19	2.7 ± 0.18	2.8 ± 0.14*	3.0 ± 0.16**	3.0 ± 0.16**	2.9 ± 0.17**
Cholesterol (mg/dL)	77.2 ± 14.30	87.8 ± 9.26	89.2 ± 22.0	92.5 ± 17.64*	100.8 ± 19.28**	106.8 ± 15.28**
SGPT (mg/dL)	28 ± 23.3	19 ± 9.9	25 ± 13.1	22 ± 6.9	16 ± 5.3**	16 ± 3.4**
SGOT (mU/mL)	71 ± 33.2	49 ± 22.4	56 ± 18.4*	51 ± 9.9*	48 ± 8.2**	48 ± 7.3**
Urea nitrogen (mg/dL)	20 ± 3.6	19 ± 4.7	23 ± 4.7	19 ± 2.3	19 ± 3.2	21 ± 4.3
Creatinine (mg/dL)	0.7 ± 0.11	0.6 ± 0.07*	0.7 ± 0.08*	0.6 ± 0.8**	0.6 ± 0.09**	0.6 ± 1.0**
GGT (mU/mL)	1 ± 0.6	1 ± 0.6	1 ± 0.2	1 ± 0.5	1 ± 0.8	2 ± 1.1**
Bilirubin (mg/dL)	0.2 ± 0.06	0.1 ± 0.05	0.2 ± 0.04	0.2 ± 0.05	0.1 ± 0.05**	0.1 ± 0.05**

*Significantly different from controls ($p < 0.05$).

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

chemistry parameters were considered to be within expected limits for this age and strain of rat.

Clinical chemistry parameters were generally comparable with controls in high-dose animals by the end of the recovery period, except that total protein ($p < 0.01$), globulin ($p < 0.001$), sodium ($p < 0.001$), and chloride ($p < 0.001$) were significantly lower in high-dose males.

7. Urinalysis: Urine was collected overnight from all animals during week 13 and from satellite animals during week 17. Food and water were removed during the collection period. The following CHECKED (X) parameters were examined:

X Volume	X Glucose
X Specific gravity	X Ketones
X pH	X Bilirubin (bile pigments)
X Sediment (microscopic)	X Blood (heme pigments)
X Protein	X Nitrate (reducing substances)
	X Urobilinogen

Results: No compound-related changes were noted.

8. Sacrifice and Pathology: All animals 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:

Digestive System	Cardiovasc./Hemat.	Neurologic
X Tongue	X Aorta[1]	XX Brain[1]
X Salivary glands[1]	X Heart[1]	X Peripheral nerve (sciatic nerve)[1]
X Esophagus[1]	X Bone marrow[1]	X Spinal cord[1] (3 levels)
X Stomach[1]	X Lymph nodes[1]	X Pituitary[1]
X Duodenum[1]	XX Spleen[1]	X Eyes[1] (optic nerve)
X Jejunum[1]	X Thymus[1]	
X Ileum[1]		Glandular
X Cecum[1]	Urogenital	XX Adrenals[1]
X Colon[1]	XX Kidneys[1]	X Lacrimal gland[1]
X Rectum[1]	X Urinary bladder[1]	X Mammary gland[1]
XX Liver[1]	XX Testes[1]	X Thyroids[1]
Gallbladder[1]	X Epididymides	X Parathyroids[1]
X Pancreas[1]	X Prostate	X Harderian glands
X Teeth	X Seminal vesicle	X Zymbals glands
	XX Ovaries	
	X Uterus[1]	
	X Vagina	

[1] Recommended by Subdivision F (November 1984) Guidelines for subchronic oral toxicity studies

	Respiratory		Other
X	Trachea[†]	X	Bone (sternum and femur)[†]
X	Lung[†]	X	Skeletal muscle[†]
X	Nasal cavity	X	Skin[†]
X	Paranasal sinuses	X	All gross lesions and masses[†]
X	Nasopharynx		
X	Middle ear		
X	Larynx and pharynx		

Results:

a. Organ Weights: Absolute and relative liver weights are summarized in Table 5. Adjusted liver weight (using body weight as a covariate) was significantly higher in males at 3000 ppm ($p < 0.05$) and females at 1500 and 3000 ppm ($p < 0.01$). These were considered to be compound related. No other compound-related changes in organ weights were noted. After the 4-week recovery period, liver weight remained slightly higher (11%) in high-dose females when compared with controls. In addition, the absolute spleen weight of high-dose females was significantly reduced ($p < 0.01$; 16%) at the end of the recovery period when compared with controls. This was not considered to be compound related because no corresponding histopathological findings were noted.

b. Gross Pathology: No compound-related abnormalities were found. Incidences of the following findings were observed with similar frequency in all groups, including controls: pale subcapsular areas in median cleft of the liver; minimal hydronephrosis; small, blue left testicle; and white nodules in the antrum mucosa of the stomach.

c. Microscopic Pathology:

1) Nonneoplastic: Microscopic liver findings are summarized in Table 6. A compound-related increase in the incidence of minimal to moderate enlargement of centrilobular hepatocytes was observed in females at 1500 (90%) and 3000 (100%) ppm. After 4 weeks of recovery, this finding was observed in 20% of the high-dose females and 5% of controls. No other compound-related findings were noted.

[†]Recommended by Subdivision F (November 1984 Guidelines for subchronic oral toxicity studies.

TABLE 5. Absolute and Relative Liver Weights (Mean ± S.D.) in a 13-Week Dietary Toxicity Study of SAN 582 H in Rats

Dietary Concentration (ppm)	Males		Females	
	Absolute[a] (g)	Relative[b] (%)	Absolute[a] (g)	Relative[b] (%)
0	23.0 ± 2.94 (22.0)	4.6 ± 0.62	10.6 ± 1.19 (10.1)	3.9 ± 0.21
50	20.8 ± 2.58 (19.2)	4.2 ± 0.44	10.1 ± 1.49 (10.0)	3.9 ± 0.50
150	22.1 ± 5.34 (20.8)	4.4 ± 0.58	10.1 ± 1.39 (10.1)	3.9 ± 0.43
500	20.8 ± 2.34 (20.4)	4.4 ± 0.33	10.8 ± 0.. (10.7)	4.2 ± 0.32
1500	20.8 ± 4.07 (22.1)	4.6 ± 0.52	11.4 ± 0.92 (11.5**)	4.5 ± 0.40**
3000	21.7 ± 2.77 (24.6*)	5.2 ± 0.56	12.4 ± 0.81 (12.9**)	5.1 ± 0.43**

[a]Statistical significance was analyzed by the study authors using body weight as a covariate. Numbers in ( ) are adjusted means.

[b]Relative (to body weight) liver weight was calculated by the reviewers using individual animal data and analyzed using Dunnett's test and ANOVA.

*Significantly different from controls ($p < 0.05$).

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

TABLE 6. Summary of Microscopic Liver Findings Observed in 13-Week Dietary Toxicity Study of SAN 582 H in Rats[a]

Findings	Exposure Level (ppm)											
	Males						Females					
	0	5	150	500	1500	5000	0	50	150	500	1500	5000
No. examined	10	10	10	10	10	10	10	10	10	10	10	10
Occasional foci of mononuclear cells	6	6	1	3	1	7	1	5	6	6	3	2
Minimal centrilobular vacuolation	3	3	0	2	1	3	0	0	0	0	0	0
Subcapsular area of fibrosis and mononuclear cells	1	0	0	0	0	0	0	0	0	0	0	0
Area of vacuolated hepatocytes	0	0	2	1	0	0	0	0	0	0	0	0
Periportal area of fibrosis/ mononuclear cells	0	0	1	0	1	1	0	0	0	0	0	0
Congested	0	0	0	1	0	1	0	0	0	0	0	0
Few sinusoidal foci of extramedullar hematopoiesis	0	0	0	0	0	0	0	0	1	0	0	0
Minimal enlargement of centrilobular hepatocytes	0	0	0	0	0	0	0	0	0	1	9	9
Moderate enlargement of centrilobular hepatocytes	0	0	0	0	0	0	0	0	0	0	0	1

[a]Microscopic findings from animals of the main study only.

2) Neoplastic: No neoplastic lesions were found.

D. STUDY AUTHORS' CONCLUSIONS:

The study authors concluded that compound-related reductions in body weight gain were observed at 500 ppm or higher. A corresponding reduction in food intake was observed at 1500 ppm or higher. Furthermore, compound-related increases in GGT at 3000 ppm and increased protein and cholesterol levels, increased liver weight, and enlargement of centrilobular hepatocytes at 1500 and 3000 ppm indicate that the liver is the primary target of toxicity. Some evidence of recovery was observed following a 4-week withdrawal from test material ingestion. A NOEL of 150 ppm was identified by the study authors.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct and reporting of this study were adequate except that a protocol was not presented. In addition, individual cage food consumption values were not reported; only average g/rat/day values were found. The concentrations of the test material in diets were within 10% of target concentrations. However, homogeneity was not demonstrated in the test diets; the study authors presented homogeneity analysis done prior to study initiation, and indicated that the method of diet preparation produced homogeneous test diets. Concentrations were not corrected for purity, but the test material was >90% pure, and therefore, correction was not necessary. Stability analysis revealed that at 3000 ppm, a decrease in test material concentration of approximately 8% was observed after 19 days of storage at room temperature in an open container (animal room conditions). However, since the test diets were prepared weekly, the reduction in stability was not considered to be a problem.

The reviewers agree with the study authors that slight, dose-related reductions in body weight were observed at 1500 and 3000 ppm. Week 13 body weights were 9 and 11% below controls in males and 5 and 10% below controls in females fed 1500 or 3000 ppm, respectively. Although dose-related, the body weight reduction in males fed 500 ppm was only 3% below controls and was not considered to be biologically significant by the reviewers.

Although the study authors reported "marginal" compound-related reductions in food consumption at 1500 ppm and higher, the reviewers were unable to assess the possible changes in food consumption because of a lack of individual cage data since animals were group-housed. This did not impact negatively on

the study, however, because toxicity was established using other parameters. The study authors considered reductions in water consumption in high-dose females to be associated with reductions in food consumption in the group. However, reductions in food consumption may also be associated with increases in water consumption, and therefore, the reviewers considered the reductions in water consumption, which averaged 14%, to be possibly related to dosing.

The reviewers agree with the study authors that the liver was the target of toxicity as indicated by increases in GGT, protein, and cholesterol levels; increases in relative liver weights; and increases in the incidence of centrilobular hepatocellular enlargement.

The reviewers agree with the study authors that there was some evidence of recovery 4 weeks after termination of dosing. However, a significant reduction in body weight and microscopic liver findings (20% incidence) were still noted in high-dose females.

Based on increased liver weights, increased protein and cholesterol, and centrilobular hepatocyte enlargement as well as decreased body weights, the LOEL and NOEL were 1500 and 500 ppm, respectively.

008285

EPA No.: 68D80056
DYNAMAC No.: 337-L
TASK No.: 3-37L
January 9, 1991

DATA EVALUATION RECORD

SAN 582H

Subchronic Oral Toxicity Study in Dogs

APPROVED BY:

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

Signature: William L. McLellan for

Date: Jan 8, 1991

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0114

DATA EVALUATION RECORD

GUIDELINE § 82-1

STUDY TYPE: Subchronic oral toxicity study in dogs.

MRID NUMBER: 416159-02.

TEST MATERIAL: SAN 582H.

SYNONYM: 2-chloro-N(2,4-dimethyl-3-thienyl N-2 methoxy)-1 methyl-acetamide.

STUDY NUMBER: IRI 3732.

SPONSOR: Sandoz AG, Basle, Switzerland.

TESTING FACILITY: Elphinstone Research Center, Inveresk Research International, Musselburgh, Scotland.

TITLE OF REPORT: SAN 582H: 13 Week Oral Toxicity Study in Dogs.

AUTHORS: Greenough, R. J. and Godurdhun, R.

REPORT ISSUED: August 30, 1990.

CONCLUSIONS:

SAN 582H was fed to groups of four male and four female beagle dogs for 13 weeks at dietary levels of 0, 100, 750, or 2000 ppm. Periportal hepatocellular vacuolation was observed in all (8/8) animals fed 2000 ppm and 2/8 animals fed 750 ppm; hepatic sinusoidal dilation was seen in 6/8 high-dose males and females and 1/2 mid-dose females. Histological liver changes correlated with increased serum alkaline phosphatase activity and cholesterol levels at the mid- and high-dose levels; absolute and relative (to body) liver weights were increased at the high dose, and relative (to body) liver weights were increased at the mid dose in males and females. Body weights and body weight gains of mid-dose females and high-dose males and females were depressed. Food consumption of one high-dose male was depressed throughout the study. There were no effects of toxicological importance at the 100-ppm dose level. Based on body weight depression and hepatic effects, the LOEL is 750 ppm and the NOEL is 100 ppm.

Classification: Core Minimum. This study satisfies the requirements of subchronic toxicity in a nonrodent (Guideline 82-1 ; however, homogeneity and stability analyses of the test diets used in this study were not provided and the concentration analyses of the low-dose diets were not within acceptable limits.

A. MATERIALS:

1. Test Compound: SAN 582H; description: brown liquid; batch No.: 8605; purity: 91.4%.

2. Test Animals: Species: dog; strain: beagle; age: 5 to 7 months at study initiation; weight: males--5.8 to 7.2 kg, females--5.3 to 6.7 kg at study initiation; source: Marshall Farms, North Rose, NY.

B. STUDY DESIGN:

1. Animal Assignment: Following a 4-week acclimation period, animals were assigned to the following test groups using a computerized randomization procedure:

Test group	Dose in diet (ppm)	Main study (13 Weeks) Males	Females
1 Control	0	4	4
2 Low (LDT)	100	4	4
3 Mid (MDT)	750	4	4
4 High (HDT)	2000	4	4

Dogs were vaccinated against canine distemper, hepatitis, leptospirosis, and parvovirus by the supplier. Following arrival at the testing laboratory, the animals were treated for removal of parasites and subjected to a detailed veterinary examination including abdominal palpation, auscultation, rectal temperature, and examination of skin and orifices. Prestudy clinical laboratory evaluations were performed. Dogs were housed individually in an environmentally controlled room with a temperature of 18°C (range of 15°C to 21°C), humidity of 60% (range of 44% to 76%), and a 12-hour light/dark cycle.

Basis of Dosage Selection: Dose levels were based on the results of a preliminary (palatability) study (IRI Project No. 635631; IRI Report No. 3634) in which dogs were dosed with 1500, 2000, or 2500 ppm SAN 582H for 14 days. Based on reduced food intake and concomitant body weight loss, the limit of palatability was determined to be 2000 ppm.

2. Diet Preparation: A concentrated premix of the diet was prepared weekly by addition of the basal diet to measured volumes of the test material. The test diets were prepared by diluting the premix with the appropriate amount of untreated diet to give the required concentrations, and blended in a Winkworth Change drum mixer for 20 minutes. Untreated diet was provided for the control animals. Representative samples of the test diets (0, 100, 750, and 2000 ppm) were analyzed for concentration at study initiation and at study weeks 6 and 12. The 100-ppm diet was analyzed for concentration weekly from weeks 2 to 5 and 7 to 11. The 100- and 2000-ppm test diets were analyzed for concentration at study week 13. Homogeneity and stability analyses of these diets were not reported; however, diets analyzed for the palatability study (IRI Report No. 3634) at dose levels of 50, 500, and 2500 ppm were found to be homogeneous and stable for 21 days.

   Results: With the exception of the 2000-ppm diet concentration analysis at study week 12, which was 12%

below nominal, 750- and 2000-ppm diets (3 to 5 intervals of analysis) were within 9% of target. The low-dose diet (100 ppm) varied 27% from nominal (13 intervals of analysis). The mean concentrations were 91.5 ± 7.2, 711 ± 22.6, and 1935.6 ± 147.6 for dietary levels of 100, 750, or 2000 ppm.

3. Food and Water Consumption: Animals received food (control or test diet of 400 g of ground S.D.S. Dog Diet A/day) and water ad libitum.

4. Statistics: Hematology, clinical chemistry, urinalysis, organ weight data, and body weight gains were analyzed for homogeneity of variance using the "F-max" test. If the variance appeared homogeneous, analysis of variance was used and pairwise comparisons were made with the Student's t-test. If variations were not homogeneous, log or square root transformations were used to stabilize variances.

5. Quality Assurance: A quality assurance statement was signed and dated July 10, 1987.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected at regular intervals during ~~each~~ day of testing for signs of morbidity or reaction to dosing.

Results: No mortalities occurred during the study. No clinical signs observed were attributed to dosing. Soft/loose feces was observed in control and dosed animals. Other observations (conjunctivitis, corneal edema, ocular discharge, hair loss), reported by the study author as incidental, were not tabulated.

2. Body Weight: Dogs were weighed weekly beginning 2 weeks prior to initiation of dosing; weighings were conducted prior to dosing.

Results: Representative body weight and body weight gain data are summarized in Tables 1 and 2. Body weights (18% reduction) and body weight gains (60% reduction) of high-dose females were significantly depressed ($p < 0.05$ and $p < 0.001$, respectively) at study week 13 when compared to concurrent controls. This depression was in part a reflection of the depressed weight of one high-dose female (animal No. 30) throughout the study period when compared to body weights of other high-dose females or concurrent control weights; however, the body weights of this animal were slightly lower (0.3 to 1.0 kg) at predose weighings when compared to other animals of the group. Food consumption of this animal was depressed from study weeks

TABLE 1. Mean Body Weights at Selected Intervals in a 90-Day Oral Toxicity Study of SAN 582H in Dogs

Dose Level (ppm)	Mean Body Weight (kg ± S.D.) at Study Weeks: 0	7	13
		Males	
0	6.5 ± 0.48	7.5 ± 0.68	8.3 ± 0.83
100	6.6 ± 0.79	7.9 ± 0.59	8.3 ± 0.69
750	6.6 ± 0.57	7.6 ± 0.78	8.3 ± 0.70
2000	6.6 ± 0.61	6.8 ± 0.78	7.2 ± 1.02
		Females	
0	6.1 ± 0.49	7.3 ± 0.42	8.0 ± 0.56
100	5.8 ± 0.52	7.1 ± 0.82	7.8 ± 0.65
750	5.7 ± 0.19	6.7 ± 0.37	7.0 ± 0.48
2000	5.8 ± 0.51	6.2 ± 0.62	6.6 ± 0.61*[a]

[a]The mean body weights of high-dose females at week 13 were reported by the study authors as nonsignificant; these data were reanalyzed by our reviewers and found to be significant at $p < 0.05$ using analysis of variance and Dunnett's test.

*Significantly different from control values at $p < 0.05$.

TABLE 2. Representative Mean Body Weight Gains in a 90-Day Oral Toxicity Study of SAN 582H in Dogs

Dose Level (ppm)	Mean Body Weight Gains (kg ± S.D.) at Study Weeks: 0-7	7-13	0-13
		Males	
0	1.0 ± 0.25	1.0 ± 0.25	2.0 ± 0.45
100	1.3 ± 0.21	0.6 ± 0.15	1.9 ± 0.18
750	1.0 ± 0.25	0.7 ± 0.36	1.7 ± 0.32
2000	0.3 ± 0.94	0.4 ± 0.24	0.7 ± 1.14**[a]
		Females	
0	1.2 ± 0.13	0.7 ± 0.26	2.0 ± 0.21
100	1.3 ± 0.33	0.7 ± 0.20	2.0 ± 0.20
750	1.0 ± 0.21	0.3 ± 0.13*	1.3 ± 0.29***
2000	0.4 ± 0.15***	0.4 ± 0.14	0.8 ± 0.15****

[a]The mean body weight gains of high-dose males during weeks 0-13 were reported by the study authors as significant at $p < 0.01$; these data were reanalyzed by our reviewers and found to be significant at $p < 0.05$ using analysis of variance and Dunnett's test.

*Significantly different from control value at $p < 0.05$.

**Significantly different from control value at $p < 0.01$.

***Significantly different from control value at $p < 0.001$.

1 to 6; the mean weight gain of this animal for the 13 weeks of the study was 0.7 kg. Body weights and bodyweight gains of mid-dose females were depressed by 13 and 37%, respectively, when compared to concurrent controls; the body weight gain was significantly ($p < 0.01$) depressed. Body weights and body weight gains of high-dose males were depressed by 15 and 65%, respectively; the body weight gain was significantly ($p < 0.05$) depressed. This depression of male weights was in part a reflection of the depressed weights of one high-dose male (animal No. 26) beginning at study week 2 and continuing throughout the study period (1 to 1.4 kg depression from predose weight). Food consumption of this animal was depressed throughout the study period; the body weight gain was depressed 1.0 kg for the 13 weeks of the study.

3. Food Consumption and Compound Intake: Food consumption was determined daily for each dog but was reported at weekly intervals.

Results: The food consumption of high-dose males and females was depressed below predose levels during study week 1. The food consumption of one high-dose male (animal No. 26) was depressed below predose levels throughout the study. Food consumption of other high-dose animals improved during the study; there were no effects of food consumption on low- or mid-dose animals. Compound intakes were not reported.

4. Ophthalmological Examinations: Ophthalmological examinations were performed prior to dosing and at study weeks 6 and 12. Anterior, lenticular, and fundic areas were examined after mydriasis resulting from application of Mydriacil®.

Results: No compound-related findings were observed. The changes seen (suture lines in the lens, hyaline remnant in the vitreous humor, pinhead opacity of the cornea, opaque cornea) were considered to be incidental background findings.

5. Hematology and Clinical Chemistry: Blood was collected from the jugular vein from fasted animals prior to study initiation and at study weeks 6 and 12 for hematology and clinical analysis. 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	Platelet count·	X	Mean corpuscular volume (MCV)
X	Reticulocyte count (RETIC)	X	Coagulation: prothrombin time (PT)
	Red cell morphology		

Results: There were no changes in hematology parameters which were considered to be a result of dosing. Values were generally within the normal reference range for the species.

b. Clinical Chemistry:

	Electrolytes		Other
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·		Globulins
		X	Glucose·
	Enzymes	X	Total bilirubin·
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase	X	Total protein·
	Creatine phosphokinase		Triglycerides
X	Lactic acid dehydrogenase	X	Protein electrophoresis
X	Serum alanine aminotransferase (SGPT)·		
X	Serum aspartate aminotransferase (SGOT)·		
	Gamma glutamyltransferase (GGT)		

Results: Changes in selected clinical chemistry parameters are presented in Table 3. Alkaline phosphatase activity of high-dose females was nonsignificantly increased at 6 weeks (78% increase when compared to concurrent controls) and

·Recommended by Subdivision F (November 1984 Guidelines for subchronic toxicity studies.

TABLE 5. Selected Mean Clinical Chemistry Parameters (Mean ± S.D.) in a 90 Day Oral Toxicity Study of SAN 582H in Dogs[a]

Parameter / Interval (Week)	Dietary Level (ppm)							
	Males				Females			
	0	100	750	2000	0	100	750	2000
Cholesterol (mmol/L)								
0	3.6 ± 0.22	4.3 ± 0.37	3.9 ± 0.92	4.1 ± 0.49	3.9 ± 0.60	4.5 ± 0.55	4.1 ± 0.88	5.0 ± 0.68
6	3.5 ± 0.59	3.9 ± 0.36	4.3 ± 1.20	4.6 ± 0.34	3.6 ± 0.34	4.1 ± 0.28	4.5 ± 1.09	6.4 ± 1.17**[b]
12	3.3 ± 0.46	3.9 ± 0.23	3.8 ± 1.07	4.2 ± 0.53	3.4 ± 0.47	3.9 ± 0.36	4.1 ± 1.26	5.4 ± 0.93*[c]
Alkaline Phosphatase (IU/L)								
0	341.5 ± 63.50	391.0 ± 99.14	304.0 ± 41.79	329.3 ± 102.01	370.5 ± 126.15	551.0 ± 353.04	307.5 ± 80.22	368.8 ± 80.86
6	316.0 ± 72.59	316.0 ± 73.15	300.3 ± 26.79	402.8 ± 110.17	337.8 ± 116.77	461.8 ± 240.46	335.5 ± 62.99	599.8 ± 236.43
12	282.5 ± 83.20	274.8 ± 75.20	287.8 ± 42.38	481.8 ± 179.23	284.8 ± 129.54	422.3 ± 219.85	302.3 ± 96.57	672.0 ± 197.48*[c]
SGPT (IU/L)								
0	16.5 ± 4.51	15.5 ± 5.51	15.0 ± 4.08	22.8 ± 16.78	21.8 ± 5.06	20.0 ± 5.48	17.8 ± 1.26	17.8 ± 5.12
6	23.5 ± 5.74	27.0 ± 7.35	20.5 ± 4.43	22.8 ± 5.32	30.8 ± 12.28	24.5 ± 4.65	21.8 ± 0.96	24.3 ± 10.21
12	22.3 ± 6.18	26.5 ± 6.45	27.0 ± 5.35	43.3 ± 37.21	37.0 ± 26.78	26.3 ± 4.99	22.3 ± 3.77	48.8 ± 35.87

[a]Values may differ slightly from those reported owing to rounding by the study authors.

[b]Mean cholesterol levels of high-dose females at 6 weeks were reported by the study authors as significant at $p < 0.001$; these data were reanalyzed by our reviewers and found to be significant at $p < 0.01$ using analysis of variance and Dunnett's test.

[c]Mean cholesterol levels and mean alkaline phosphatase levels of high-dose females at 12 weeks were reported by the study authors as significant at $p < 0.01$; these data were reanalyzed by our reviewers and found to be significant at $p < 0.05$ using analysis of variance and Dunnett's test.

*Significantly different from control values at $p < 0.05$.

**Significantly different from control values at $p < 0.01$.

significantly ($p < 0.05$) increased at 12 weeks (136% increase when compared to concurrent controls); alkaline phosphatase activity of high-dose males was nonsignificantly increased at 6 (27% increase) and 12 weeks (71% increase) when compared to concurrent controls. This increase was dose related in males and females and became more elevated with time.

Cholesterol levels were significantly increased in high-dose females at 6 weeks ($p < 0.01$, 78% increase) and 12 weeks ($p < 0.05$, 59% increase); cholesterol levels of high-dose males were slightly but not significantly increased at 6 weeks only (31% increase when compared to concurrent controls but only 12% increase when compared to pretest level). SGPT levels were slightly increased at 12 weeks in high-dose males and females; this resulted from the increased SGPT level of one high-dose male (animal No. 25) and female (animal No. 31). Without these outliers, the mean SGPT levels of high dose males and females would be 24.7 ± 2.31 and 31.3 ± 10.5 IU/L, respectively. Albumin levels were significantly ($p < 0.01$) decreased in high-dose males at study weeks 6 and 12; however, the changes were slight (9 to 11%). albumin levels in dosed females were similar to those of controls, and slight decreases in albumin are of questionable toxicological significance in the beagle dog.

Urinalysis: Urine was collected from fasted animals over 17 hours of a 21-hour water-deprivation period. Fecal samples were collected and examined for the presence of occult blood at the time of urine collection. The CHECKED (X) parameters were examined:

	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

Results: There were no effects of biological importance on the urinalyses of dosed animals. Slightly increased specific gravity in dosed animals was sporadic and not associated with increased urine output; the study authors did not consider this finding to be a result of dosing. Occult blood analysis of feces was negative for all animals.

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; the (XX) organs were weighed. Additional sections of formalin-fixed liver and kidney were stained with Oil Red O to determine the presence of fat.

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

Results:

a. Organ Weights: Table 4 presents data for mean liver weights and liver-to-body weight ratios. Mean absolute liver weights of high-dose males and females were significantly ($p < 0.01$) increased (25 and 32% in males and females, respectively) when compared to concurrent controls. Liver weights of mid-dose males and females were slightly but nonsignificantly increased (13% males, 6% females). Liver-to-body weight ratios were

Recommended by Subdivision F (November 1984) Guidelines for subchronic toxicity studies.

TABLE 4. Absolute and Relative Liver Weights (Mean ± S.D.) in a 90-Day Oral Toxicity Study of SAN 582H in Dogs[a]

Dose Group (ppm)	Males		Females	
	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)
0	260.5 ± 21.52	3.1 ± 0.27	237.4 ± 22.64	3.0 ± [illegible]
100	280.0 ± 22.14	3.3 ± 0.22	237.5 ± 12.86	3.2 ± [illegible]
750	294.1 ± 18.62	3.6 ± 0.26*	252.6 ± [illegible]	[illegible]
2000	325.5 ± 30.39**	4.6 ± 0.27**	312.5 ± 20.68**	4.8 ± [illegible]**

[a]Organ-to-body weight ratios were calculated by our reviewers and statistically analyzed using analysis of variance.

*Significantly different from control values at $p < 0.05$.

**Significantly different from control values at $p < 0.01$.

recalculated by the reviewers and are presented in Table 4. Liver-to-body weight ratios were significantly increased in mid- ($p < 0.05$) and high-dose ($p < 0.01$) males and females; ratios were increased by 16 and 48% in males and 20 and 60% in females, respectively.

Other changes in organ weights (adrenals, thyroids, thymus and lungs in males, pituitary in females) were considered to be incidental based on the lack of corresponding pathological change, decreased terminal body weights, absence of dose-response relationships, or absence of similar changes in animals of the opposite sex.

b. Gross Pathology: Macroscopic lesions were sporadic in dosed and control animals. The most frequent finding was intestinal roundworms observed in one control female, one low-dose male, three low-dose females, two mid-dose males, four mid-dose females, four high-dose males, and three high-dose females. Reddened intestinal mucosa was observed in several animals. Mild liver lesions (described as irregular areas of prominent lobulation) were observed in one high-dose male and female; these liver lesions were considered by the study authors to be related to dosing. Summary data were not provided.

c. Microscopic Pathology: Table 5 presents representative histological findings. Periportal hepatocellular vacuolation was observed in 4/4 high-dose males and females and 1/4 mid-dose males and females. The severity of the lesions was not graded. The vacuoles did not contain lipid when stained with Oil Red O, and no evidence of glycogen was found. Sinusoidal dilation was also found in the liver of 3/4 high-dose males and females and 1/4 mid-dose females. These hepatocellular findings are considered to be related to dosing. Other histopathological findings were considered to be incidental. A summary table was provided for liver lesions only.

D. STUDY AUTHORS' CONCLUSIONS:

The 13-week dietary administration of SAN 582H to male and female beagle dogs at dose levels of 0, 100, 750, or 2000 ppm resulted in lower body weight gains in mid-dose males and high-dose males and females. Food consumption was depressed in few high-dose animals during study week 1 only. Alkaline phosphatase and cholesterol levels were increased in high-dose males and females. Absolute and relative liver weights were

TABLE 5. Histopathological Lesions of the Liver Observed in a 90-Day Oral Toxicity Study of SAN 582H in Dogs

Finding	Dietary Level (ppm)							
	Males				Females			
Number of animals examined:	0 (4)	100 (4)	750 (4)	2000 (4)	0 (4)	100 (4)	750 (4)	2000 (4)
Periportal hepatocellular vacuolation	0	0	1	4	0	0	1	4
Sinusoidal dilation	0	0	0	3	0	0	1	3

increased in high-dose animals; relative liver weights were increased at the mid-dose level. Hepatocytic vacuolation and dilation of liver sinusoids occurred in mid- and high-dose animals. The LOEL is 750 ppm, and the NOEL is 100 ppm SAN 582H.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate and the conduct of the study was acceptable; however, homogeneity and stability analyses of the test diets used in this study were not provided. Even though diets at dose levels of 50, 500, and 2500 ppm were analyzed and reported for the palatability study, these doses were not used for the palatability or subchronic studies; analyses of the test material in the diet should be conducted for each study prior to the initiation of that study on dose levels used in the study. In addition, the concentration analysis of the low-dose diet (100 ppm) varied 27% from nominal, which is not within acceptable limits. Various body weight, body weight gain, and clinical chemistry data were reevaluated by our reviewers, resulting in levels of significance for these parameters that differed from those reported by the study authors; changes in data are noted in the appropriate tables.

We agree with the study authors that the primary effects of SAN 582H in dogs of this study were depressed body weights, increased alkaline phosphatase and cholesterol levels, increased liver weights, and hepatocytic vacuolation and dilation of hepatic sinusoids. The LOEL is 750 ppm, and the NOEL is 100 ppm SAN 582H.

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

008285

EPA No.: 68D80056
DYNAMAC No.: 337-M
TASK No.: 3-37M
January 11, 1991

DATA EVALUATION RECORD

SAN 582H

52-Week Oral Toxicity Study in Dogs

APPROVED BY:

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

Signature: Robert J. Weir

Date: 1-10-91

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008285

EPA No.: 68D80056
DYNAMAC No.: 337-M
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January 11, 1991

DATA EVALUATION RECORD

SAN 582H

52-Week Oral Toxicity Study in Dogs

REVIEWED BY:

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Date: Jan 11, 1991

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008285

DATA EVALUATION RECORD

GUIDELINE § 83-1

STUDY TYPE: Oral toxicity study in dogs.

MRID NUMBER: 416159-03.

TEST MATERIAL: SAN 582H.

SYNONYM: 2-chloro-N(2,4 dimethyl-3 (thienyl) N-2 methoxy)-1 methyl-acetamide.

STUDY NUMBER(S): Report No. 5242 and IRI Project No. 635579.

SPONSOR: Sandoz AG, Basle, Switzerland.

TESTING FACILITY: Inveresk Research International, Musselburgh, Scotland.

TITLE OF REPORT: SAN 582H - 52 Week Oral Toxicity Study in Dogs.

AUTHOR: Greenough, R. J.; Goburdhun, R; and MacNaughtan, F.

REPORT ISSUED: August 30, 1990.

CONCLUSIONS: SAN 582H was fed to groups of four male and four female beagle dogs for 1 year at dietary levels of 0, 25, 250, or 1250 ppm (approximately 0, 2, 9.6, or 49 mg/kg/day, respectively). At the highest dose tested, a variable degree of periportal hepatocyte vacuolation was seen in 2/4 males and 4/4 females. The only other finding in the liver was minimum or mild midzonal hepatocyte enlargement in two males and one female receiving 1250 ppm. The liver changes at the high-dose correlated with an increase in serum alkaline phosphatase activity and cholesterol levels and increased liver-to-body weight ratios in both sexes.

Body weight gains were decreased (nonsignificantly) in high-dose males and females, but these decreases were of doubtful toxicologic importance. There were no effects of toxicologic importance at dose levels of 25 or 250 ppm. We conclude that the LOEL was 1250 ppm (49 mg/kg/day) and the NOEL was 250 ppm (9.6 mg/kg/day). The dogs may have been able to tolerate a slightly higher dose.

CORE Classification: This study satisfies the Core Guideline requirements for chronic toxicity in a nonrodent species (Guideline 83-1).

A. MATERIALS:

1. Test Compound: SAN 582H; description: brown liquid; batch No.: 8605; purity: 91.3%.

2. Test Animals: Species: dog; strain: purpose-bred Beagle; age: 4 to 4.5 months at receipt, 5-6 months at initiation; weight at receipt: males--5.5 to 7.2 kg, females--5.2 to 6.5 kg; source: Marshall Farms, North Rose, NY.

B. STUDY DESIGN:

1. Animal Assignment: Dogs were assigned to the following groups using random numbers and a balanced distribution, taking body weights and litter mates into account.

Test group	Dose in diet (ppm)	Main study (12 months)	
		Males	Females
1 Control	0	4	4
2 Low (LDT)	50	4	4
3 Mid (MDT)	250	4	4
4 High (HDT)	1250	4	4

Dogs had been vaccinated against canine distemper, hepatitis, leptospirosis, and parvovirus. They were treated with antihelmintics following arrival, acclimated for 6 weeks, and subjected to a detailed veterinary examination including abdominal palpation, auscultation, rectal temperature, and evaluation of the skin and orifices. Pretrial clinical laboratory analyses were performed. Dogs were housed singly in 1 x 2 m pens with an automatic water dispenser in a room with temperature at 18°C (10-26°C transient extremes) and 56% humidity (transient extremes 30-86%) and a 12 hour light/dark cycle.

2. Diet Preparation: A premix was prepared by adding weighed quantities of untreated ground diet to measured volumes of test compound, adding the premix to additional diet, and mixing for 20 minutes in a drum mixer. Batches of diet were prepared weekly, and 100-g samples of freshly mixed diets at each dose level were retained frozen. Three 50-g samples from all groups were taken at initiation and at approximately 2-month intervals for analysis of concentration, homogeneity, and stability.

   Results: With the exception of the high-dose formulation at week 1, which was 36% below nominal, and the low-dose diet at week 1, which was 12% below nominal, all other diets analyzed (seven intervals: 2, 6, 9, 17, 25, 39 and 46 weeks) were within 8% of target. The mean measured concentrations were 49.9 ± 6, 243 ± 9.5, and 1,222 ± 29 ppm at nominal dietary concentrations of 50, 250, or 1,250 ppm, respectively. Recovery after storage at room temperature for 21 days was 90% for the 50-ppm diet. For other data on stability, previous studies were referenced. The coefficients of variance for homogeneity were between 3.1 and 3.3% for 50-ppm diets at two intervals of analysis.

3. Food and Water Consumption: Each dog was offered 400 g dry diet/day (SDS Diet A ground, Special Diet Services LTD Witham, Essex. Food was offered in the morning and left with each dog until the following morning when the residue was weighed. Water was available ad libitum.

4. Statistics: Hematology, clinical chemistry, and body weight gain data were analyzed for homogeneity of variance using the "F-max" test. If the group variance appeared homogeneous, a parametric ANOVA was used and pairwise comparisons were made with Student's T-test. If variances were not homogeneous, log or square root transformations were used to stabilize variances. Organ weights were also analyzed by covariance; body weight was the covariate.

5. Quality Assurance: A quality assurance statement was signed and dated February 24, 1989, and a GLP compliance statement was present.

C. METHODS AND RESULTS:

1. Observations: The dogs were observed at regular intervals during each working day for signs of ill health or reaction to dosing. The nature, time of onset, severity, and duration of findings were recorded.

Results: No mortalities occurred during the study. No clinical signs of toxicity related to dosing were observed. The main clinical finding was occasional soft/loose stools. The frequency of the finding was similar in dosed and control groups. The total number of observations of the finding/dose group each week on study was tabulated. No other data on observations were provided, and no individual animal findings (in-life) were recorded on pathology sheets.

2. Body Weight: Animals were weighed weekly (prior to feeding) beginning 2 weeks before initiation of dosing. Terminal body weights were recorded immediately prior to sacrifice.

Results: Representative body weight data are summarized in Table 1. There were no significant decreases in mean body weights or weight gains comparing dosed groups and controls. Transient fluctuations in body weight for individual animals were seen for several dosed dogs; the changes were not considered to be of toxicologic importance for mature dogs. An apparent decrease (nonsignificant) in weight gain in male dogs receiving 250 and 1250 ppm was not considered to be of toxicologic importance. Male No. 18 (250 ppm) had a net weight loss of 0.4 kg; no effect on food consumption was seen, and the gain from weeks 1 through 26 (+0.4 kg) was normal. Male No. 27 (1250 pm) lost 0.5 kg during the study; weight gain for the first 26 weeks was 1.0 kg, and a sharp weight loss from week 49 to 50 (-0.7 kg) was accompanied by slightly decreased food consumption.

3. Food Consumption and Compound Intake: Food consumption was recorded daily for each dog, and weekly food consumption was calculated and recorded for individual dogs.

Weekly group mean values for compound consumption (mg/kg/day) were calculated based on nominal dietary concentrations, actual body weight, and food consumption data.

TABLE 1. Mean Body Weights and Weight Gains at Selected Intervals in Dogs Fed SAN 582H for 52 Weeks

Dietary Level (ppm)	Mean body weight (kg ± S.D.) at Week: 0	13	26	52	Mean weight gain (kg ± S.D.) between weeks[a] 0-26	26-52
			Males			
0	7.8 ± 1.31	9.3 ± 1.97	9.9 ± 1.82	10.5 ± 1.29	2.2 ± 0.59	0.5 ± 0.65
50	7.9 ± 0.21	9.4 ± 0.51	10.0 ± 0.65	10.1 ± 0.80	2.1 ± 0.47	0.2 ± 0.54
250	7.9 ± 0.49	9.1 ± 1.28	9.6 ± 1.49	9.6 ± 2.11	1.7 ± 1.03	0.03 ± 0.62
1250	7.8 ± 0.49	8.5 ± 0.64	8.7 ± 0.67	8.7 ± 0.93	0.9 ± 0.53	0.0 ± 1.07
			Females			
0	6.6 ± 0.87	8.0 ± 1.09	8.2 ± 1.01	9.1 ± 0.95	1.6 ± 0.48	0.9 ± 0.38
50	6.4 ± 0.56	8.0 ± 0.96	8.6 ± 1.53	9.2 ± 1.11	2.2 ± 1.04	0.6 ± 0.66
250	6.6 ± 0.48	8.0 ± 0.54	8.4 ± 1.07	8.9 ± 1.15	1.8 ± 0.84	0.5 ± 0.29
1250	6.6 ± 0.60	7.5 ± 1.27	7.5 ± 1.40	8.3 ± 1.36	2.8 ± 0.63	1.0 ± 0.27

[a]Calculated by our reviewers.

Results: No adverse effects of dosing on food consumption were seen. A few individual animals sporadically consumed less than 50% of their daily food ration. Mean consumption values for females dosed at 1250 ppm were lower than pretest values. The mean corresponding consumptions at dietary levels of 50, 250, and 1250 ppm were 1.95, 10.1, or 48.7 mg/kg/day in males and 2.1, 9.1, or 49.3 mg/kg/day in females, respectively.

4. Ophthalmological Examinations: Both eyes of all dogs were examined prior to dosing and at 26 and 51 weeks using an indirect ophthalmoscope. Anterior, lenticular, and fundic areas were evaluated after mydriasis with one drop of 1% Mydracil[5].

Results: No treatment-related findings were observed. A few abnormalities that were seen were typical background findings (hyaline remnant in the vitreous humor, suture lines in lens, ulceration of the cornea).

5. Hematology and Clinical Chemistry: Blood was collected from the jugular vein prior to dosing, and during weeks 13, 26, and 51 from dogs fasted overnight. The CHECKED (X) parameters were examined:

a. Hematology:

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

Results: No changes in any parameters that were related to dosing were observed. For all parameters, values were generally within the normal range for the species. Low platelet counts occasionally seen were attributed to aggregation in blood smears. Platelets counts were significantly increased ($p < 0.01$) in high-dose males at week 26, but the mean value was well within the normal range (350×10^3/mL) whereas the mean value for controls was lower than normal.

[5]Recommended by Subdivision F (November 1984) Guidelines.

b. Clinical Chemistry:

	Electrolytes		Other
X	Calcium[1]	X	Albumin[1]
X	Chloride[1]	X	Albumin/globulin ratio
	Magnesium[1]	X	Blood creatinine[1]
	Phosphorus[1]	X	Blood urea nitrogen[1]
X	Potassium[1]	X	Cholesterol[1]
X	Sodium[1]		Globulins
		X	Glucose[1]
	Enzymes	X	Total bilirubin[1]
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase	X	Total protein[1]
	Creatine phosphokinase[1]		Triglycerides
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)[1]		
X	Serum aspartate aminotransferase (SGOT)[1]		
X	Gamma glutamyltransferase (GGT)		

Results: Treatment-related effects on serum cholesterol levels and alkaline phosphatase activity were reported for high-dose males and females (Table 2). Alkaline phosphatase activity was significantly increased in high-dose females at 13, 26, and 51 weeks, and there was an apparent positive dose trend at weeks 13 and 26. Alkaline phosphatase was increased in high-dose males at weeks 26 and 51, and the increase was significant compared to controls at week 26 ($p < 0.01$). Cholesterol levels were increased in high-dose males at weeks 13 and 26, and the increase was significant ($p < 0.01$) at 26 weeks. The mean cholesterol level was increased in high-dose males and females at 13 weeks, but the increases were not significant compared to controls and all values were within the range for concurrent controls (3.8 to 7.1 mmol/L). An increase in GGT activity was observed at week 13 in a high-dose female (No. 32); the same female had elevated SGOT and SGPT levels at 26 weeks. There were no changes in mean values of SGOT or SGPT that were consistent with time or dose.

6. Urinalysis: Urinalyses were performed prior to dosing and during weeks 13, 26, and 51. The CHECKED (X) parameters were examined:

X	Appearance[1]	X	Glucose[1]
X	Volume[1]	X	Ketones
X	Specific gravity[1]	X	Bilirubin[1]
X	pH	X	Blood[1]
X	Sediment (microscopic)[1]		Nitrate
X	Protein[1]	X	Urobilinogen

[1]Recommended by Subdivision F (November 1984) Guidelines.

TABLE 2. Selected Mean Clinical Chemistry Values (Mean ± S.D.) in a 52-Week Oral Toxicity Study of SAN 5824 in Dogs

Parameter/ Interval (Week)	Dietary Level (ppm)							
	Males				Females			
	0	50	250	1250	0	50	250	1250
Alkaline Phosphatase (IU/L)								
0	332 ± 98	294 ± 78	309 ± 75	258 ± 46	284 ± 55	292 ± 40	310 ± 45	368 ± 64
13	190 ± 49	157 ± 39	180 ± 56	196 ± 60	162 ± 40	168 ± 24	186 ± 48	421 ± 163***
26	141 ± 38	150 ± 44	167 ± 44	254 ± 51**	134 ± 42	164 ± 100	206 ± 89	426 ± 220**
51	133 ± 38	139 ± 45	162 ± 62	196 ± 71	145 ± 41	140 ± 30	140 ± 38	303 ± 92**
Cholesterol (mmol/L)								
0	3.9 ± 0.8	3.6 ± 0.4	3.6 ± 0.4	3.5 ± 0.5	4.1 ± 0.9	3.4 ± 0.3	3.4 ± 0.8	4.2 ± 0.4
13	3.1 ± 0.6	3.6 ± 0.3	3.7 ± 1.0	4.1 ± 1.0	3.9 ± 0.1	3.3 ± 0.5	4.4 ± 1.3	5.5 ± 1.5
26	3.0 ± 0.3	3.5 ± 0.2	3.6 ± 0.6	4.7 ± 1.3**	5.2 ± 1.4	4.7 ± 1.0	5.2 ± 0.5	4.9 ± 0.6
51	2.7 ± 0.1	3.1 ± 0.2	3.4 ± 0.9	3.0 ± 0.6	4.5 ± 1.5	3.8 ± 0.9	4.5 ± 0.5	5.1 ± 0.8

**Significantly different from control value, $p < 0.01$.

***Significantly different from control value, $p < 0.001$.

Results: No compound-related effects were observed.

7. Sacrifice and Pathology: All animals sacrificed at the end of the study received a complete necropsy examination. The CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

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

Results:

a. Organ Weights: Data for mean liver weights and liver-to-body weight ratios are presented in Table 3. The study authors reported that following covariance analyses with terminal body weight, there were significant increases in liver weights in males receiving 1250 ppm ($p < 0.05$), and females receiving 50 ppm ($p < 0.01$), 250 ppm ($p < 0.05$), or 1250 ppm

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 3. Absolute and Relative Liver Weights (Mean ± S.D.) in a 52-Week Oral Toxicity Study of SAN 582H in Dogs[a]

Dietary Level (ppm)	Males		Females	
	Organ Weight (g)	Organ/Body Weight (%)	Organ Weight (g)	Organ/Body Weight (%)
0	316.3 ± 28.81	3.1 ± 0.13	259.7 ± 24.94	2.9 ± 0.34
50	285.8 ± 12.97	2.9 ± 0.17	313.6 ± 33.84	3.5 ± 0.12*
250	295.7 ± 40.05	3.2 ± 0.70	290.9 ± 29.96	3.3 ± 0.19
1250	343.5 ± 51.97	4.0 ± 0.26*	309.9 ± 31.12	3.8 ± 0.30**

[a]Organ-to-body weight ratios were calculated by our reviewers and statistically analyzed by ANOVA.

*Significantly different from control value, $p < 0.05$.

**Significantly different from control value, $p < 0.01$.

(p <0.01). They did not consider the increases at 50 and 250 ppm (females) to be of toxicologic importance, since there were no correlating clinical chemistry or pathology changes or any dose-related trend. Analysis of liver-to-body weight data by our reviewers indicated a significant increase in males receiving 1250 ppm (p <0.05) and in females receiving 50 ppm (p <0.05) or 1250 ppm (p <0.01), but no significance in females at 250 ppm when compared to controls.

b. Gross Pathology: Gross lesions were sporadic and infrequent, and none were related to dosing. The most frequent finding was intestinal roundworms (found in one control female and a male and female in both the 50- and 250-ppm groups). Two high-dose males had a "creamy" area in one liver lobe, and pituitary cysts were seen in a control female and one male and one female receiving 50 ppm. Summary data were not provided.

c. Microscopic Pathology:

Table 4 summarizes histologic findings. A variable degree of periportal hepatocyte vacuolation was present in high-dose animals only, affecting 2/4 males and 4/4 females. The finding ~~was graded minimal in one male~~ and one female, mild in one dog of each sex, and moderate in four females. The vacuoles did not contain lipid when stained with Oil Red O, and there was no evidence of glycogen in livers of two dogs stained with PAS. The findings indicate a hydropic degenerative change. Minimal/mild midzonal hepatocyte enlargement was also present in one high-dose female and two high-dose males. There was no evidence of any cellular damage, but cytoplasm was less dense than normal.

All other histologic findings were incidental and typical for control beagle dogs. No neoplastic findings were seen.

D. STUDY AUTHORS' CONCLUSIONS:

Chronic oral administration of SAN 582H to beagle dogs induced a mild hepatotoxic effect in the animals receiving 1250 ppm. A dose level of 1250 ppm (approximately 50 mg/kg/day) was shown to be the appropriate high dose that could be administered in view of the reduced body weight gain. Blood enzyme levels and liver weight increases correlated with hepatic pathology.

TABLE 4. Representative Histologic Findings in Dogs Fed SAN 582H for 12 Months[a]

Organ/Finding	Dietary Level (ppm)							
	Males				Females			
	0	25	250	1250	0	25	250	1250
Liver								
Periportal hepatocyte vacuolation	0	0	0	2	0	0	0	4*
Hepatocyte hypertrophy (midzonal)	0	0	0	2	0	0	0	1
Focal inflammation	3	4	4	2	3	4	3	2
Lungs								
Localized interstitial pneumonia	3	3	1	1	2	1	2	2
Focal alveolitis	1	0	2	0	1	2	0	2
Small granuloma	0	0	0	0	0	1	0	0
Testes (epididymis)								
Lymphoid foci	0	2	1	0	--	--	--	--
Pituitary								
Cysts anterior lobe	1	2	1	1	1	2	0	0
Tongue								
Inflammation lamina propria	0	0	1	0	0	2	1	0
Duodenum								
Mucosal cysts	2	2	1	1	3	2	0	2

[a]Based on examination of tissues from four dogs/sex/group.

*Significantly different from control incidence, $p < 0.05$.

The mid-dose level of 250 ppm (approximately 10 mg/kg/day) was asscciated with a reduction in body weight gain in the males and increases in liver weights in the females. There were, however, no changes in blood enzyme level or liver pathology. A dose level of 50 ppm (approximately 2 mg/kg/day) resulted only in increases in liver weight in the females.

The authors reported that the NOEL "based on blood chemistry changes and liver pathology was considered to be in the region of 50-250 ppm SAN 582H."

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct and reporting of the study were adequate. We assess that the effects in body weights and weight gains were of doubtful toxicologic importance, considering the individual animal variations seen. The dogs were close to maturity at initiation, so the weight gains expected were only in the order of 2 kg. A rather slight variation in weight gain in kg, therefore, would appear large when expressed on the basis of weight gain in the control group. The dogs may have been able to tolerate a higher dose in view of the mild histologic liver changes and slight liver weight changes. The dose was based on a previous subchronic study in dogs at 100, 750, and 2000 ppm (DER 33.7 L). In the subchronic study, mild hepatotoxic effects were seen in all dogs at 2000 ppm, and in some dogs at 750 ppm; intracellular fluid accumulation in the liver sinusoids was also seen in 75% of the high-dose males and females. Liver weights were significantly increased ($p < 0.01$) in males and females receiving 2000 ppm and a dose-related increase in liver-to-body weight ratios was seen at 750 and 2000 ppm for both sexes. Alkaline phosphatase activity was increased in both sexes at 2000 ppm at 6 and 12 weeks, but the increase was significant ($p < 0.01$) only for high-dose females. Body weight gains were 0.6 and 0.8 kg lower than controls in males and females receiving 2000 ppm.

Based on results of the 12-month study, the LOEL is 1250 ppm (99 mg/kg/day) and the NOEL is 250 ppm (9.5 mg/kg/day).

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

008285

EPA No.: 68D80056
DYNAMAC No.: 337-N
TASK No.: 3-37N
January 7, 1991

DATA EVALUATION RECORD

SAN 582H

Developmental Toxicity Study in Rats

STUDY IDENTIFICATION: Lochry, E.A. Developmental toxicity (embryo/fetal toxicity and teratogenic potential) study of SAN 582H administered orally via gavage to Crl:COBS CD (SD)BR presumed pregnant rats. (Unpublished study No. 1319-001 conducted by Argus Research Laboratories, Inc., Perkasie, PA, and submitted by Sandoz Agrodevelopment, Basel, Switzerland; dated July 23, 1987.) MRID No. 416159-04.

APPROVED BY:

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

Signature: Robert J. Weir

Date: 1/7/91

0145

008285

1. CHEMICAL: SAN 582H.

2. TEST MATERIAL: SAN 582H, batch No. not reported, 98.3% pure, described as a viscous liquid.

3. STUDY/ACTION TYPE: Developmental toxicity study in rats.

4. STUDY IDENTIFICATION: Lochry, E.A. Developmental toxicity (embryo/fetal toxicity and teratogenic potential) study of SAN 582H administered orally via gavage to Crl:COBS CD (SD)BR presumed pregnant rats. (Unpublished study No. 1319-001 conducted by Argus Research Laboratories, Inc., Perkasie, PA, and submitted by Sandoz Agrodevelopment, Basel, Switzerland; dated July 23, 1987.) MRID No. 416159-04.

5. REVIEWED BY:

Pia Lindstrom, DPH
Principal Reviewer
Dynamac Corporation

Signature: Pia Lindstrom
Date: January 7, 1991

Patricia Turck, M.S.
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Date: January 18, 1991

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0146

DATA EVALUATION RECORD

STUDY TYPE: Developmental toxicity. Guideline §83-3.

MRID NUMBER: 416159-04.

TEST MATERIAL: SAN 582H.

SYNONYMS: None reported.

STUDY NUMBER: 1319-001.

SPONSOR: Sandoz Agrodevelopment, Basel, Switzerland.

TESTING FACILITY: Argus Research Laboratories, Inc., Perkasie, PA.

TITLE OF REPORT: Developmental Toxicity (Embryo/Fetal Toxicity and Teratogenic Potential) Study of SAN 582H Administered Orally via Gavage to Crl:COBS CD (SD)BR Presumed Pregnant Rats.

AUTHOR: Lochry, E.A.

REPORT ISSUED: July 23, 1987.

CONCLUSIONS: A developmental toxicity study was conducted in which Crl:COBS CD (SD)BR rats were administered SAN 582H via gavage at 0, 50, 215, and 425 mg/kg/day during gestational days (GD) 6 through 15. Maternal toxicity, evidenced by excess salivation, increased liver weight, and reduced body weight gain and food consumption during the dosing period, was observed in the mid- and high-dose groups. Based on these results, the maternal NOEL and LOEL were 50 and 215 mg/kg/day, respectively.

Developmental toxicity, evidenced by an increased incidence of resorptions, was observed in fetuses from dams in the high-dose group. Therefore, the developmental toxicity NOEL and LOEL were 215 and 425 mg/kg/day, respectively.

Classification: CORE Minimum Data. This study meets the minimum requirements set forth under Guideline §83-3 for a developmental toxicity study in rats.

A. MATERIALS:

Test Compound:	Purity:	Approximately 98.3%.
	Description:	Viscous liquid.
	Lot No.:	Not reported.
	Contaminants:	Not reported.
Vehicle:	Hi-Sil 233 (lot No. I.13.6.A, PPG Industries, Inc.) and 0.5% (w/v) aqueous carboxymethyl cellulose (lot No. 114F-0414, Sigma Chemical Co.).	
Test Animals:	Species:	Rat.
	Strain:	Crl:COBS CD (SD)BR.
	Source:	Charles River Breeding Laboratories, Inc., Raleigh, NC.
	Age:	71 days at receipt.
	Weight:	212-299 g on GD 0.

B. STUDY DESIGN:

This study was designed to assess the potential of SAN 582H 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 and source (73 days old, weighing 283-345 g at receipt) 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: Females were randomly assigned to dose groups using a computer-generated (weight-ordered) randomization procedure based on GD 0 body weights as follows:

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control	0	25
Low dose	50	25
Mid dose	215	25
High dose	425	25

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 daily from a dry powder consisting of approximately 50% SAN 582 H and 50% Hi Sil-233 (w/w). This was done to achieve a free-flowing wettable powder, which was then suspended in 0.5% aqueous carboxymethyl cellulose. The powder was prepared by combining equal volumes of test substance and Hi-Sil 233 with three times the volume of acetone and then allowing the solution to dry overnight (i.e., the acetone evaporated). Homogeneity and stability analyses were performed in the preliminary dose range-finding study. Concentration analyses were conducted three times during the study (randomly chosen) to include all dose levels.

The selection of doses was based on two preliminary studies. In the first study, the test material was administered to groups of 10 mated rats via gavage on GD 6-15 at dose levels of 0, 16, 32, 60, 130, or 260 mg/kg/day. At 130 and 260 mg/kg/day, slight reductions in maternal food consumption and body weight gain were observed, while at 260 mg/kg/day, fetal body weight was slightly decreased. In the second study, the test material was administered via gavage to groups of five nonpregnant rats at dose levels of 0, 400, and 600 mg/kg/day for seven days, and animals were sacrificed 24 hours after the last dose. At 400 and 600 mg/kg/day, clinical signs of toxicity, body weight loss, and increased absolute and relative liver weights were observed. At 600 mg/kg/day, increased mortality (60%) and decreased food consumption were also noted.

Observations: Animals were observed twice daily for mortality. In addition, they were observed several times daily during the treatment and posttreatment periods for overt signs of toxicity. Body weight and food consumption were recorded on GD 0 and 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;
- Number and placement of implantation sites; and
- Number of early and late resorptions and live and dead fetuses.

Any gross lesions found during necropsy were preserved in 10% neutral buffered formalin for possible histopathologic evaluation.

Fetuses were examined in the following manner:

- Individual fetuses were weighed and sexed;
- External alterations were recorded;
- Approximately half of the fetuses in each litter were 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), maternal body weight change for GD 16-20, 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, 0-20, 6-9, 6-12, 9-12, 12-16, 6-16, 6-19, 6-20--Analysis of co-variance;
- 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 May 3, 1990, was provided.
- A signed Statement of Compliance with EPA GLP's, dated August 30, 1990, was provided.
- A signed Quality Assurance Statement, dated July 23, 1987, was provided.

C. RESULTS:

The following results were reported by the study author.

1. Dose Analysis: Homogeneity of the test substance in the carrier/vehicle was confirmed; analysis revealed that the maximum difference between samples was 5%. Dose suspensions from a preliminary range-finding study were analyzed for stability. After 26 days under refrigeration, test material concentrations were -21.7 and -11.4% of initial concentrations (1.87 and 26.1 mg/mL, respectively). Analyses of the dosing suspensions used in the main study revealed concentrations ranging from 92 to 101% of the target concentrations (5.0, 21.5, and 42.5 mg/mL).

2. Maternal Toxicity:

Mortality: Two pregnant control animals were found dead on GD 16. Necropsy revealed that one animal died of an intubation error, while the other exhibited dark red dried substance on hindpaws and around the vaginal opening, a pale liver, and dark red or brown viscous material in the stomach, vagina, and cervix.

Abortion: No abortions occurred during this study.

Clinical Observations: A summary of clinical observations is presented in Table 1. The number of dams exhibiting excess salivation was significantly ($p \leq 0.01$) increased above controls in mid- and high-dose groups. The incidences of thin appearance and urine-stained abdominal fur were significantly ($p \leq 0.01$) increased in the high-dose group.

Body Weight: A summary of maternal body weight gain is presented in Table 2. Body weight in GD 12 high-dose animals was significantly ($p \leq 0.01$) decreased compared with the control group (data not shown). Body weight gain was significantly ($p \leq 0.01$ or ≤ 0.05) decreased in mid- and

TABLE 1. Clinical Observations[a]

Observation:	Dose Level (mg/kg/day) 0	50	215	425
Maximum possible incidence/No. of rats:	357/25	373/25	375/25	375/25
Chromorrhinorrhea	3/1	0/0	0/0	1/1
Chromodacryorrhea	2/1	0/0	0/0	0/0
Thin appearance	0/0	0/0	0/0	3**/2
Red exudate - vagina	1/1	0/0	1/1	1/1
Excess salivation	0/0	2/2	31/20**	63**/20**
Rales	1/1	0/0	0/0	0/0
Urine - stained abdominal fur	1/1	0/0	0/0	4**/2
Localized alopecia	13/2	0**/0	12/2	15/2

[a]Data were extracted from study No. 1319-001, Table 2.

**Significantly different from controls ($p \leq 0.01$).

TABLE 2. Mean Body Weight Gain (g ± 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	35.0 ± 6.6	57.1 ± 10.4	67.3 ± 8.8	160.0 ± 18.6
50	37.0 ± 7.9	51.8 ± 9.8	67.6 ± 11.2	156.5 ± 22.6
215	36.7 ± 7.5	47.8 ± 13.1**	68.2 ± 12.1	152.7 ± 22.6
425	39.0 ± 6.4	37.2 ± 12.2**	67.0 ± 12.2	143.2 ± 17.3**

[a]Data were extracted from study No. 1319-001, Table 4.

**Significantly different from controls ($p \leq 0.01$).

high-dose animals for GD 6-9 and for the dosing period (GD 6-16), and in high-dose animals for GD 6-12, GD 9-12, GD 6-19, GD 6-20, and for the entire gestational period (GD 0-20).

Food Consumption: A summary of food consumption data is presented in Table 3. During the dosing period (GD 6-16), food consumption (g/animal/day) was significantly decreased for mid-dose animals ($p \leq 0.05$) as well as for high-dose animals ($p \leq 0.01$). Food consumption calculated as g/kg/day (data not shown) showed a similar pattern; reduced food consumption was observed during the first half of the dosing period and was comparable to controls for the remaining gestational period.

Gross Pathological Observations: A summary of absolute and relative liver weights is presented in Table 4. Hydronephrosis was noted in one nonpregnant high-dose animal. Absolute maternal liver weight was significantly ($p \leq 0.05$ or 0.01) increased in the mid- and high-dose groups, while relative liver weight was significantly ($p \leq 0.05$ or 0.01) increased in the low-, mid-, and high-dose groups.

Cesarean Section Observations: A summary of cesarean section data is presented in Table 5. One high-dose animal had 100% resorptions. The number of resorptions was nonsignificantly increased in mid- and high-dose groups. The number of live fetuses was nonsignificantly decreased in the high-dose group.

3. Developmental Toxicity:

A summary of external, visceral, and skeletal alterations is presented in Table 6.

External Examinations: Two low-dose fetuses (separate litters) exhibited external malformations; one fetus had a tail that was thread-like at the tip, and the other had a bleb on the head.

Visceral Examinations: One fetus from the control group exhibited agenesis of the intermediate and diaphragmatic lobes of the lungs with related displacement of the heart (malformations). One fetus from the mid-dose group displayed hydrocephalus (malformation). Dilation of the renal pelvis (variation) was evident in one fetus from the high-dose group.

Skeletal Examinations: The following malformations were observed: cervical rib in five control fetuses (one

TABLE 3. Mean Food Consumption (g/animal/day ± 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	21.8 ± 1.6	24.4 ± 1.6	26.6 ± 2.0	24.0 ± 1.4
50	22.1 ± 2.6	23.7 ± 2.2	26.7 ± 2.3	23.8 ± 2.2
215	21.8 ± 2.3	22.9 ± 2.1*	27.3 ± 2.4	23.4 ± 1.9
425	22.6 ± 1.6	22.3 ± 2.6**	26.8 ± 2.6	23.3 ± 1.6

[a]Data were extracted from study No. 1319-001, Table 5.

*Significantly different from controls ($p \leq 0.05$).

**Significantly different from controls ($p \leq 0.01$).

TABLE 4. Absolute and Relative Liver Weights[a]

Parameter	Dose Level (mg/kg/day) 0	50	215	425
Absolute liver weight	16.82 ± 1.45	17.80 ± 1.56	17.94 ± 1.56*	19.31 ± 1.88**
Relative liver weight	4.06 ± 0.27	4.32 ± 0.41*	4.39 ± 0.29**	4.82 ± 0.43**

[a]Data were extracted from study No. 1319-001, Table 3.

*Significantly different from controls ($p \leq 0.05$).

**Significantly different from controls ($p \leq 0.01$).

TABLE 5. Cesarean Section Observations[a]

Parameter	Dose Level (mg/kg/day) 0	50	215	425
No. animals assigned	25	25[b]	25	25[c]
No. animals pregnant	24	25	23	23
Pregnancy rate (%)	96	100	92	92
Maternal wastage				
No. nonpregnant	1	0	2	2
No. pregnant dead	2	0	0	0
Total corpora lutea[d]	386	418[b]	419	426
Corpora lutea/dam	17.5	17.4	18.2	17.6
Total implantations[d]	349	378[b]	374	369
Implantations/dam	15.9	15.8	16.3	16.0
Total live fetuses	335	355	342	320
Live fetuses/dam	15.2	14.8	14.9	13.9
Total resorptions[d]	14	23	32	49[c]
Early	14	21	32	47
Late	0	2	0	2
Resorptions/dam	0.6	1.0	1.4	2.1
Total dead fetuses	0	0	0	0
Fetal weight/litter (g)	3.67	3.67	3.63	3.60
Preimplantation loss (%)[e]	9.3	10.8	10.1	13.8
Postimplantation loss (%)[f]	3.8	6.2	9.0	14.6*
Sex ratio (% male)	47.9	49.9	47.7	49.4

[a]Data were extracted from study No. 1319-001, Tables 7, 8, and 18.

[b]One dam was excluded from calculations because of mistimed pregnancy.

[c]One litter was completely resorbed.

[d]Calculated by reviewers using individual animal data.

[e]Calculated by reviewers as $\frac{\text{(No. corpora lutea - No. implants)/litter}}{\text{No. corpora lutea/litter}} \times 100$ using ANOVA and linear regression analyses.

[f]Calculated by reviewers as $\frac{\text{(No. implants - No. live fetuses)/litter}}{\text{No. implants/litter}} \times 100$ using ANOVA and linear regression analyses.

*Significantly different from controls ($p < 0.05$).

TABLE 6. Summary of Fetal Alterations[a]

Findings[b]	Dose Level (mg/kg/day) 0	50	215	425
External Alterations:				
No. fetuses (litters) examined	335 (22)	355 (24[c])	342 (23)	320 (22[d])
Head, bleb	0	1	0	0
Tail, thread-like	0	1	0	0
Total No. fetuses (litters) affected	0	2 (2)	0	0
Visceral Alterations:				
No. fetuses (litters) examined	160 (22)	172 (24)	167 (23)	154 (22)
Hydrocephalus	0	0	1	0
Lungs, intermediate and diaphragmatic lobes, agenesis	1	0	0	0
Heart, displacement	1	0	0	0
Kidney, pelvis, moderate dilation	0	0	0	1
Total No. fetuses (litters) affected	1	0	1	1
Skeletal Alterations:				
No. fetuses (litters) examined	175 (22)	183 (24)	175 (23)	166 (22)
Frontals, contained holes	0	1	0	0
Fontanelle, anterior and posterior, enlarged and irregularly shaped	0	1	0	0
Interparietals, incompletely ossified	0	1	0	0
Cervical rib present	5 (1)	0**	1**	1**
Thoracic centra, bifid	0	1	0	1
Ribs, short	0	0	0	2 (1)
Manubrium, incompletely ossified	1	0	0	2 (2)
Pelvis, pubis and/or ischia, incompletely ossified	2 (2)	1	0	0
Rear limb, femur, bent	0	0	0	1
Sternebrae, incompletely or not ossified	6 (4)	4 (2)	2 (2)	3 (3)
Sternebrae, asymmetric	1	0	0	0
Total No. fetuses (litters) affected	12 (5)	8 (6)	3 (3)	8 (6)

[a]Data were extracted from study No. 1319-201, Tables 10, 11, 12, and 21.

[b]More than one type of alteration may be found in one fetus.

[c]One litter excluded because of mistimed pregnancy.

[d]One dam had 100% resorptions.

**Significantly different from controls ($p \leq 0.01$).

litter), one mid-dose fetus, and one high-dose fetus; asymmetric sternebrae in one control fetus; bent femur in one high-dose fetus; and short rib in two high-dose fetuses (one litter). Variations (incomplete ossification, bifid thoracic centra, and frontals with small holes) were observed in all groups with similar frequencies (see Table 5 for details).

D. REVIEWERS' DISCUSSION/CONCLUSIONS:

1. Dose Analyses: The analyses conducted on two samples from the range-finding study to confirm the stability of the test material suspensions revealed greater variability than is commonly accepted. Nominal concentrations of the samples tested were 1.87 and 26.1 mg/mL. On day 0, concentrations found were 1.57 (84%) and 24.5 (94%) mg/g; on day 26 they were 1.91 (102%) and 21.7 (83%) mg/g.

2. Maternal Toxicity: Maternal toxicity was evidenced in mid- and high-dose groups during the dosing period and included a significant increase in the number of animals displaying clinical signs of toxicity (excess salivation), significantly increased absolute and relative liver weights, and significantly reduced body weight gain and food consumption. A statistically significant increase in ~~relative liver weight was also observed at 50 mg/kg/day~~. However, since no other toxicity was observed at 50 mg/kg/day, the apparent dose-related response was not considered to be biologically significant. The reduced body weight gain occurred during the first part of the dosing period, but was comparable to the control group during the second half of the dosing period.

   Based on these results, the maternal NOEL and LOEL were 50 and 215 mg/kg/day, respectively.

2. Developmental Toxicity:

   a. Deaths/Resorptions: Due to 100% resorptions confirmed in one animal from the high-dose group, the number of resorptions/dam increased to 2.1. Although the increase was not statistically significant, it was outside the range of historical control data (0.3-1.4). Postimplantation loss (calculated by the reviewers) was significantly increased in the high-dose group as a result of a small decrease in live fetuses/dam (control: 15.2 versus high-dose: 13.9).

   b. Altered Growth: No compound-related effects were observed.

c. Developmental Alterations: The observed fetal alterations (malformations and variations) were notconsidered to be attributed to the test material. They were either single events occurring in a non-dose-dependent manner, they occurred in all groups at similar frequencies, or they were within the range of historical controls.

Developmental toxicity was evidenced by an increased incidence in resorptions. Increases, although not statistically significant, were observed at all dose levels, but were outside the range of historical control data for the high-dose group only. In addition, the number of dams with resorptions (9, 15, 16, and 18 in the control, low-dose, mid-dose, and high-dose groups, respectively) appeared to increase in a dose-related manner.

Based on these results, the developmental NOEL and LOEL were 215 and 425 mg/kg/day, respectively.

3. Study Deficiencies:

a. No protocol was submitted.

b. No gravid uterine weight was recorded; therefore, ~~corrected weight gain could not be calculated.~~

c. No statistical analysis was performed on pre- and postimplantation losses. These have been provided by the reviewers.

E. CLASSIFICATION: CORE Minimum Data.

Maternal NOEL = 50 mg/kg/day.
Maternal LOEL = 215 mg/kg/day.
Developmental Toxicity NOEL = 215 mg/kg/day.
Developmental Toxicity LOEL = 425 mg/kg/day.

F. RISK ASSESSMENT: Not applicable.

008285

EPA: 68D80056
DYNAMAC No.: 337-O
TASK No.: 3-370
January 7, 1991

DATA EVALUATION RECORD

SAN 582H

Two-Generation Reproductive Toxicity Study in Rats

STUDY IDENTIFICATION: Suter, P., K. Biedermann, J. Wilson, and Ch. Terrier. Two-generation reproduction study with SAN 582 H in the rat. (Unpublished study No. 201205 conducted by Research and Consulting Company (RCC) AG and RCC Umweltchemie AG, Itingen, Switzerland, and submitted by Sandoz Ltd., Basel, Switzerland; dated May 17, 1990.) MRID No. 416159-05.

APPROVED BY:

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

Signature: Robert J. Weir

Date: 1/7/91

1. CHEMICAL: SAN 582H.

2. TEST MATERIAL: SAN 582H, batch No. 8710, 92.6% pure, brown viscous liquid.

3. STUDY/ACTION TYPE: Two-generation reproduction study in rats.

4. STUDY IDENTIFICATION: Suter, P., K. Biedermann, J. Wilson, and Ch. Terrier. Two-generation reproduction study with SAN 582 H in the rat. (Unpublished study No. 201205 conducted by Research and Consulting Company (RCC) AG and RCC Umweltchemie AG, Itingen, Switzerland, and submitted by Sandoz Ltd., Basel, Switzerland; dated May 17, 1990.) MRID No. 416159-05.

5. REVIEWED BY:

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008285

DATA EVALUATION RECORD

STUDY TYPE: Reproductive Toxicity. Guideline §83-4.

MRID NUMBER: 416159-05.

TEST MATERIAL: SAN 582H.

SYNONYM(S): None reported.

STUDY NUMBER: 201205.

SPONSOR: Sandoz Ltd., Basel, Switzerland.

TESTING FACILITY: Research and Consulting Company (RCC) AG and RCC Umweltchemie AG, Itingen, Switzerland.

TITLE OF REPORT: Two-Generation Reproduction Study with SAN 582 H in the Rat.

AUTHORS: Suter, P., K. Biedermann, J. Wilson, and Ch. Terrier.

REPORT ISSUED: May 17, 1990.

0163

CONCLUSIONS: In a two-generation reproductive toxicity study in which groups of rats were administered SAN 582H at concentrations of 0, 100, 500, or 2000 ppm (approximately 7, 36, or 150 mg/kg/day for males and 8, 40, or 160 mg/kg/day for females) in the diet for two consecutive generations, parental toxicity, as evidenced by significant reductions in body weight and food consumption in males and significant increases in absolute and relative liver weights in both males and females, was observed at 2000 ppm. The NOEL and LOEL for parental toxicity were 500 and 2000 ppm, respectively.

No changes in pregnancy rates, fertility, or length of gestation were observed. Significant reductions in pup weight during lactation were noted at 2000 ppm. Therefore, the NOEL and LOEL for reproductive toxicity were 500 and 2000 ppm, respectively.

Classification: CORE Minimum Data. This study meets the minimum requirements set forth under Guideline 83-4 for a reproductive toxicity study in rats.

A. MATERIALS:

Test Compound: Purity: 92.6%.
Description: Dark brown, viscous liquid.
Batch No.: 8710.
~~Contaminants: Not reported.~~

Vehicle(s): A vehicle was not used; the test material was administered in the diet. Acetone was the carrier solvent.

Test Animals: Species: Rat.
Strain: Wistar/HAN (Kfm:WIST, outbred, SPF).
Source: Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland.
Age: Approximately 3 weeks at study initiation.
Weight: P males, 171-221 g; P females, 131-162 g; F_1 males, 50-209 g; F_1 females, 131-162 g at study initiation.

B. STUDY DESIGN:

This study was designed to assess the reproductive toxicity potential of SAN 582H, when administered continuously in the diet for two consecutive generations.

Mating: After 70 days on the test diets, P animals were paired one male:one female from the same dietary group for a maximum 21 days. Day 0 of gestation was designated as the day on which

sperm in the vaginal smear or a copulatory plug was found. Several P females (a total of 32) were remated with the same males for additional time (length of second pairing not reported) when evidence of mating from the first pairing was questionable. The F_1 parents were randomly chosen at weaning and were fed test diets for 101 days prior to pairing. The mating procedure was the same as described for the P generation. Sibling pairings were avoided.

Group Arrangement: P parents were randomly assigned to groups using a computer-generated random algorithm; F_1 parents were randomly chosen, but the method was not reported.

Test Group	Dietary Concentration (ppm)	Number Assigned per Group P M	P F	F_1 M	F_1 F
Control	0	25	25	25	25
Low dose	100	25	25	25	25
Mid dose	500	25	25	25	25
High dose	2000	25	25	25	25

Dosing: The test material was administered continuously in the diet for two consecutive generations. At least every 2 weeks, the test material was dissolved in acetone and mixed with powder feed. The mixture was then pelleted, and the pellets were dried for 48 h in warm air prior to being stored at room temperature until use. Test material concentration in the diet was analyzed three times during each generation. Homogeneity was also determined at three mixing intervals during each generation. Stability of the test material in feed had been determined in a previous study and was confirmed prior to study initiation.

Dietary levels were chosen based on the results of a preliminary one-generation reproduction pilot study (RCC Project No. 201194), but the details and results were not reported.

Observations: Animals were observed twice daily for mortality and overt signs of toxicity. Body weights and food consumption were recorded weekly during the premating period. After mating, females were weighed on gestational days (GD) 0, 7, 14, and 21 and on lactational days 1, 4, 7, 14, and 21. Males were

TABLE 1. Summary of Body Weights During the Premating Period for Rats Fed SAN 582 H for Two Consecutive Generations[a]

Dietary Concentration (ppm)	Mean Body Weight (g ± S.D.) at Study Week: 2	5	8	11
P Males				
0	230 ± 16	297 ± 22	344 ± 26	377 ± 27
100	229 ± 11	295 ± 18	342 ± 22	374 ± 24
500	231 ± 13	297 ± 24	343 ± 30	377 ± 37
2000	221 ± 11*	283 ± 19	322 ± 23*	352 ± 20*
P Females				
0	165 ± 11	200 ± 14	218 ± 17	227 ± 18
100	164 ± 9	198 ± 12	217 ± 13	228 ± 15
500	161 ± 8	196 ± 11	213 ± 12	223 ± 14
2000	162 ± 5	193 ± 17	209 ± 10*	219 ± 11
F1 Males				
0	174 ± 40	298 ± 36	368 ± 34	411 ± 36
100	189 ± 36	305 ± 31	369 ± 31	409 ± 35
500	184 ± 33	300 ± 27	362 ± 28	401 ± 29
2000	161 ± 40	274 ± 33*	338 ± 32*	373 ± 33*
F1 Females				
0	137 ± 24	193 ± 19	218 ± 19	235 ± 19
100	147 ± 18	200 ± 16	230 ± 18	244 ± 20
500	143 ± 21	196 ± 19	226 ± 19	241 ± 20
2000	132 ± 23	188 ± 21	212 ± 23	230 ± 23

[a]Data were extracted from study No. 201305, pp. 66, 70, 94, and 99.

*Significantly different from controls ($p < 0.05$).

observed on days 50 and 57 (weeks 8-9) of the premating period. Body weight and body weight gain (not shown) were comparable among P females from control and all test groups during the gestation and lactation periods.

During the F_1 generation, consistent, significant reductions ($p < 0.05$) in body weight were noted in high-dose males during both the premating (after day 29) and postmating periods. Body weight and body weight gain were similar among F_1 females from the control and test groups throughout the study period.

Food Consumption: Food consumption during the premating period is summarized in Table 2. Significant reductions ($p < 0.05$) in food consumption of high-dose P males were observed on days 36-57 (weeks 6-8) and 64-70 (week 10) of the premating period and days 8-23 of the postmating period. For P females, significant reductions ($p < 0.05$) in food consumption were noted on days 22-29 (week 4) and 50-57 (week 8) at the high dose and on days 43-50 (week 6) at the mid dose. Significant increases ($p < 0.05$) were observed on days 15-22 and 57-64 (weeks 3 and 6, respectively) at the low dose. Generally, food consumption was 5-6% below control levels in high-dose P females during the premating period. During gestation (data not shown), a 6.3% reduction (not statistically significant) was seen on GD 0-7, and during days 4-14 postpartum, a 6-8% reduction (not statistically significant) was observed.

In the F_1 generation, food consumption of high-dose males was significantly reduced ($p < 0.05$) on days 1-8, 15-50, and 57-92 (weeks 1, 3-7, and 8-13, respectively) of the premating period and days 15-22 of the postmating period. In mid-dose F_1 males, significant reductions ($p < 0.05$) were noted on days 57-64, 85-92, and 99-101 (weeks 9, 13, and 15, respectively). In F_1 females, food consumption was significantly higher than controls for 100-ppm females on days 43-57 (weeks 7-8) and for 500-ppm females on days 36-43 and 50-57 (weeks 6 and 8, respectively). Food consumption during gestation and lactation were similar between controls and test groups (data not shown).

Relative food consumption (g/kg/day) was generally similar between control and test groups during both generations.

Mean values for test material intake of the low-, mid-, and high-dose P and F_1 males ranged from 5-12, 24-63, and 98-270 mg/kg/day, respectively, during the study. For P and F_1 females, mean test material intake ranged from 6-8, 32-92, and 130-387 mg/kg/day for low-, mid-, and high-dose groups, respectively, during the premating and gestation periods.

TABLE 2. Summary of Food Consumption (g/animal/day) During the Premating Period for Rats Fed SAN 582 H for Two Consecutive Generations[a]

Dietary Concentration (ppm)	Mean Food Consumption (± S.D.) at Study Week: 1	4	7	10
P Males				
0	19.8 ± 1.8	22.7 ± 1.8	21.9 ± 2.0	21.4 ± 1.9
100	19.9 ± 1.7	22.2 ± 1.9	21.6 ± 1.8	21.4 ± 1.8
500	19.8 ± 1.5	22.6 ± 2.2	21.2 ± 2.1	21.4 ± 2.5
2000	19.9 ± 1.5	21.5 ± 1.7	19.8 ± 1.6*	19.8 ± 1.7*
P Females				
0	14.5 ± 1.2	17.1 ± 1.3	15.5 ± 1.3	15.1 ± 1.7
100	15.1 ± 1.2	17.7 ± 1.5	16.0 ± 1.8	15.4 ± 1.2
500	14.4 ± 1.1	16.9 ± 1.5	14.5 ± 1.1*	14.9 ± 1.3
2000	14.3 ± 0.9	16.2 ± 0.9*	14.6 ± 0.8	14.4 ± 1.0
F_1 Males				
0	20.0 ± 2.8	25.3 ± 1.9	25.3 ± 1.5	25.3 ± 1.9
100	19.3 ± 2.1	25.1 ± 1.7	23.8 ± 2.1	24.4 ± 2.4
500	19.5 ± 2.0	24.9 ± 2.0	24.1 ± 1.9	24.3 ± 2.1
2000	18.3 ± 2.8*	23.4 ± 2.0*	23.6 ± 2.0*	23.6 ± 2.0*
F_1 Females				
0	15.5 ± 1.9	17.6 ± 1.4	16.9 ± 1.4	17.0 ± 1.3
100	15.3 ± 1.9	18.1 ± 1.9	18.3 ± 1.3*	17.7 ± 2.1
500	15.2 ± 2.4	17.9 ± 1.8	17.7 ± 1.5	17.7 ± 1.7
2000	15.1 ± 2.1	16.9 ± 2.1	16.8 ± 1.6	16.7 ± 1.6

[a]Data were extracted from study No. 201205, pp. 54, 58, 78, and 83.

*Significantly different from controls ($p < 0.05$).

weighed, and food consumption was measured weekly after the mating period. Food consumption of females was measured on GD 0, 7, 14, and 21 and on lactational days 1, 4, 7, and 14.

The following data were recorded for each litter:

- Litter size, number of stillborn and live pups, and external abnormalities on the day of birth (day 0 postpartum);
- Individual pup weight on days 0 (or 1), 4, 7, 14, and 21 postpartum;
- Sex ratio on days 0, 4, and 21 postpartum; and
- Survival and behavioral abnormalities daily.

On day 4 postpartum, litters were randomly culled to eight pups (four/sex, if possible). Culled pups were then killed and subjected to a gross necropsy. Pups not chosen as F_1 parents and all F_2 pups were killed and subjected to gross necropsy at weaning on day 21 postpartum.

Carcasses of pups were preserved in buffered 4% formaldehyde solution. Dead pups were necropsied and/or preserved for possible future examination. All P and F_1 adults were killed when they were "no longer necessary for assessment of reproductive effects" and subjected to a gross necropsy. Liver weight was recorded for all P and F_1 adults. The following tissues were collected and preserved:

-Gross lesions
-Liver
-Ovaries
-Pituitary gland
-Uterus* and cervix
-Prostate
-Seminal vesicles with coagulating gland
-Testes with epididymides
-Vagina

*The uteri were first stained in aqueous ammonium sulfide solution to detect implantation sites.

The above organs from control and high-dose parents, reproductive organs from all infertile males and females, and all gross lesions were histologically examined.

Statistical Analysis: The following statistical analyses were conducted.

- Parental and pup body weight, parental food consumption, organ and organ-to-body weight ratios--Univariate ANOVA and Dunnett's test.
- Spontaneous pup mortality--Fisher's Exact test.
- Reproductive data--Wilcoxon ranks, Univariate ANOVA, and Kruskall-Wallis test.

Compliance:

- A signed Statement of No Data Confidentiality Claim, dated May 27, 1990, was provided.
- A signed Statement of Compliance with EPA GLP's, dated August 30, 1990, was provided.
- A signed Quality Assurance Statement, dated May 18, 1990, was provided.

C. RESULTS:

The study authors reported the following results.

1. Test Material Analysis: Mean dietary concentrations ranged from 92.6 to 105.3% of target concentrations, and homogeneity analysis revealed that aliquots varied from +4 to -2% of mean concentrations. The test material was stable in feed for over 21 days at room temperature.

2. Parental Toxicity:

Mortality: No deaths were observed in either generation.

Clinical Observations: Isolated incidences of red areas on tail, hair loss, hemorrhagic spots on the abdomen, and bleeding from the vagina were observed in all dietary groups including controls.

Body Weight: Premating body weight data are summarized in Table 1. Significant reductions ($p < 0.05$) in body weight were observed in high-dose P males on days 8-22 (weeks 2-4) and 36-70 (weeks 6-11) of the premating period and on days 1 and 8 of the postmating period. In addition, a 12% reduction in body weight gain for the premating period was noted in high-dose P males. In P females, statistically significant reductions ($p < 0.05$) in body weight were

Gross Pathological Observations: The following findings were noted at necropsy with similar frequency among all groups, including controls, in both generations: enlarged, swollen or discolored liver, hepatic nodule, enlarged coagulating gland, small (unilateral) seminal vesicles, cystic ovaries, distended or dilated uterine horn, discolored kidneys, subcutaneous nodule, and alopecia. Histological examination revealed a mammary adenocarcinoma in one low-dose female, and severe seminiferous tubular atrophy was observed in one mid-dose male who failed to sire a litter.

Terminal body weight and absolute and relative (to body weight) liver weight data are summarized in Table 3. Significant increases ($p < 0.01$) in both absolute and relative liver weight were observed in high-dose males and females from both generations. In addition, slight increases in relative liver weight were observed in mid-dose P males and F_1 males and females; a significant increase ($p < 0.05$) was observed in mid-dose P females.

3. Reproductive Toxicity: The effects of dietary administration of the test material on reproductive parameters are summarized in Table 4. No changes in pregnancy rate, fertility indices, or length of gestation were observed in either generation. Slight, but nonsignificant, reductions in implantions (data not shown), total number of pups born, mean litter size at birth, and mean viable pups/litter on days 0 and 4 postpartum were observed in the mid- and high-dose P groups when compared with controls. Postimplantation loss was slightly increased (nonsignificant) in the high-dose F_1 group.

Significant reductions ($p < 0.05$) in body weight were observed in high-dose F_1 pups on days 14 and 21 postpartum. Significant increases ($p < 0.05$) in F_1 pup body weight were observed at the low dose throughout the lactation period and at the mid dose on days 1-7 postpartum. During the F_2 generation, pup body weight in the high-dose group was significantly reduced ($p < 0.05$) on days 7-21 postpartum. A significant decrease ($p < 0.05$) was also observed in the mid-dose F_2 group on day 7 postpartum.

One control F_1 pup did not have a tail. In the F_2 generation, two control pups had bent lateral tails.

TABLE 3. Absolute and Relative Liver Weight Data for Rats Fed SAN 582 H for Two Consecutive Generations[a]

Parameter	Dietary Concentration (ppm)							
	0		100		500		2000	
	M	F	M	F	M	F	M	F
P Generation								
Body weight (g)	415	277	419	275	421	273	393	267
Absolute liver weight (g)	13.58	12.10	13.29	12.41	14.38	13.14	15.14**	14.38**
Relative liver weight (%)	3.28	4.35	3.15	4.48	3.42	4.79*	3.85**	5.39**
F1 Generation								
Body weight (g)	495	287	492	293	473	294	454**	287
Absolute liver weight (g)	16.48	12.93	15.83	12.85	16.23	13.80	18.08**	15.59**
Relative liver weight (%)	3.33	4.50	3.21	4.39	3.43	4.69	3.99**	5.43**

[a]Data were extracted from study No. 201205, pp. 146-153.

*Significantly different from controls ($p < 0.05$).

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

Gross Pathological Observations: The following were noted at necropsy with similar frequency ar groups, including controls, in both generations: e: swollen or discolored liver, hepatic nodule, (coagulating gland, small (unilateral) seminal v cystic ovaries, distended or dilated uterin discolored kidneys, subcutaneous nodule, and a Histological examination revealed a mammary adenoc in one low-dose female, and severe seminiferous atrophy was observed in one mid-dose male who fa sire a litter.

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Significant reductions ($p < 0.05$) in body weig observed in high-dose F_1 pups on days 14 and 21 pos Significant increases ($p < 0.05$) in F_1 pup body wei observed at the low dose throughout the lactation and at the mid dose on days 1-7 postpartum. During generation, pup body weight in the high-dose gro significantly reduced ($p < 0.05$) on days 7- postp A significant decrease ($p < 0.05$) was also observed mid-dose F_2 group on day postpartum.

One control F_1 pup did not have a tail. In generation, two control pups had bent lateral tai

TABLE 3. Absolute and Relative Liver Weight Data for Rats Fed SAN 582 H for Two Consecutive Generations[a]

Parameter	Dietary Concentration (ppm)							
	0		100		500		2000	
	M	F	M	F	M	F	M	F
P Generation								
Body weight (g)	415	277	419	275	421	273	393	267
Absolute liver weight (g)	13.58	12.10	13.29	12.41	14.38	13.14	15.14**	14.38**
Relative liver weight (%)	3.28	4.35	3.15	4.48	3.42	4.79*	3.85**	5.39**
F1 Generation								
Body weight (g)	495	287	492	293	473	294	454**	287
Absolute liver weight (g)	16.48	12.93	15.85	12.85	16.23	13.80	18.08**	15.59**
Relative liver weight (%)	3.33	4.50	3.21	4.39	3.43	4.69	3.99**	5.43**

[a]Data were extracted from study No. 201205, pp. 146-153.

*Significantly different from controls ($p < 0.05$).

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

D. REVIEWERS' DISCUSSION/CONCLUSION:

1. Test Material Analysis: Analysis of test material concentration, homogeneity, and stability revealed that the test material was adequately stable in feed under conditions of the study, the test diets were homogeneous, and actual concentrations were within acceptable range of target concentrations.

2. Parental Toxicity: Significant, compound-related reductions in body weight and food consumption were observed in P and F. males fed 2000 ppm. Absolute and relative liver weights were significantly increased in both P and F_1 males and females fed 2000 ppm. No corresponding microscopic changes were observed in the livers of high-dose animals. Slight increases (6-10%) in absolute and relative liver weight were also observed in mid-dose P animals, and relative liver weight was slightly increased (3-4%) in low-dose P females and mid-dose F_1 males and females. However, although the increases in relative liver weight appeared to be dose related, the increases were not significant, corroborating microscopic changes were not observed in high-dose animals (histopathological examination of the livers from mid-dose animals was not performed), and no other evidence of toxicity was seen at the mid-dose level. Therefore, the apparent dose-related increase in relative liver weight alone was not considered to be an adverse effect.

Based on significant reductions in body weight and food consumption in high-dose parental males, and significant increases in absolute and relative liver weight in both high-dose males and females, the parental NOEL and LOEL were 500 and 2000 ppm, respectively.

3. Reproductive Toxicity: No adverse effects on pregnancy rates, fertility indices, or length of gestation were observed. Body weights of pups from the 2000-ppm group were significantly decreased during the latter part of lactation, beginning on day 14 for the first generation and day 7 for the second generation.

Reductions in number of implantations (data not shown), postimplantation loss, total pups/litter, and viable pups/litter were observed in the high-dose group during the first generation. Although the number of implantations and postimplantation loss observed in high-dose animals were

008285

EPA No.: 68D80056
DYNAMAC No.: 337-G
TASK No.: 3-37G
January 4, 1991

DATA EVALUATION RECORD

SAN 582H

Mutagenicity--*Salmonella typhimurium*/Mammalian Microsome Mutagenicity Assay

APPROVED BY:

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Signature: Robert J. Weir

Date: 1/3/91

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Guideline Series 84: **Mutagenicity**
EPA No.: 68D80056
DYNAMAC No.: 337-G
TASK No.: 3-37G
January 4, 1991

DATA EVALUATION RECORD

SAN 582H

Mutagenicity--*Salmonella typhimurium*/Mammalian Microsome Mutagenicity Assay

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Salmonella

DATA EVALUATION RECORD

Tox. Chem. No.:
EPA File Symbol:

CHEMICAL: SAN 582H.

STUDY TYPE: Salmonella/mammalian activation gene mutation assay.

MRID NUMBER: 415965-42.

SYNONYMS/CAS NUMBER. None listed.

SPONSOR: Sandoz Crop Protection Corp., Des Plaines, IL.

TESTING FACILITY: Hazleton Laboratories America, Inc., Kensington, MD.

TITLE OF REPORT: Mutagenicity Test on SAN 582H in the Ames Salmonella/Microsome Reverse Mutation Assay.

AUTHORS: Haworth, L. and Lawlor, T. E.

STUDY NUMBER: 10767-0-401.

REPORT ISSUED: July 31, 1989.

CONCLUSION(S) - Executive Summary: SAN 582H was evaluated for the potential to cause gene mutations in two independently performed Salmonella typhimurium/mammalian microsome plate incorporation mutagenicity assays. The six nonactivated doses ranged from 50 to 6,500 µg/plate, and the six S9-activated doses ranged from 100 to 10,000 µg/plate. Concentrations ≥2,500 µg/plate -/-S9 precipitated, and cytotoxicity was apparent for the majority of strains exposed to the highest nonactivated (6,500 µg/plate) and the highest S9-activated (10,000 µg/plate) dose. There was

however, no appreciable increase in mutant colonies of any strain at any dose either with or without S9 activation.

Based on these findings, we assess that an appropriate range of test material doses was evaluated, and that SAN 582H was not mutagenic in this test system. The study, therefore, fulfills the Guideline requirements for Category I, Gene Mutations.

A. MATERIALS:

1. Test Material:

Name:	SAN 582H, technical
Description:	Viscous, dark brown liquid
Lot No.:	8605
Purity:	91.4%
Contaminants:	Not listed
Solvent used:	Dimethylsulfoxide (DMSO)
Other comments:	The test material was stored at room temperature in the dark and formed a solution at 200 mg/mL in DMSO. Solutions of the test material were freshly prepared on the day of use.

2. Control Materials:
Negative: None
Solvent/final concentration: DMSO/50 µL/plate
Positive: Nonactivation:
Sodium azide 2.0 µg/plate TA100, TA1535
2-Nitrofluorene 1.0 µg/plate TA98, TA1538
ICR-191 2.0 µg/plate TA1537
Other:

Activation:
2-Aminoanthracene 2-anthramine) 2.5 µg/plate all strains.

3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The S9 liver homogenate was purchased from an unspecified commercial source.

S9 mix composition:	Amount/mL
Sodium phosphate buffer	100.0 µmol
Glucose 6-phosphate	5.0 µmol
NADP	4.0 µmol
$MgCl_2$	8.0 µmol
KCl	33.0 µmol
S9	100.0 µL

4. Test Organism Used: S. typhimurium strains
_____ TA97 __x__ TA98 __x__ TA100 _____ TA102 _____ TA104
__x__ TA1535 __x__ TA1537 __x__ TA1538; List any others:

Test organisms properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

a. Preliminary cytotoxicity assay: Ten doses (10, 33.3, 66.7, 100, 333, 667, 1,000, 3,330, 6,670, and 10,000 ~~ug/plate) were evaluated~~ with or without S9 activation in S. typhimurium strain TA100. Single plates were used per dose per condition.

b. Initial Mutation assay: Six nonactived doses (50, 100, 500, 1,000, 2,500, and 5,000 ug/plate) and six S9-activated doses (100, 500, 1,000, 2,500, 5,000, and 10,000 ug/plate) were evaluated in all tester strains.

c. Confirmatory mutation assay: As above for the initial mutation assay with and without S9 activation in all tester strains.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay: __x__ Standard plate test
_____ Pre-incubation (___) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (describe).

2. Protocol:

a. Plating procedures: In general, similar procedures were used for the preliminary cytotoxicity and the mutation assays.

To tubes containing 2.5-mL volumes of molten top agar 100 µL of an overnight broth culture of the appropriate tester strain and 50 µL of the appropriate test material dose, solvent, or positive controls were added. For the S9-activated test, 0.5 mL of the S9 cofactor mix was added to tubes containing 2.0 mL of top agar; tester strains and test and control solutions were added as described. The contents of the tubes were mixed, poured over Vogel-Bonner minimal medium E, and incubated at 37°C for ≈48 hours. At the end of incubation, plates either were immediately scored for revertant colonies or were refrigerated and subsequently counted with an automatic colony counter. Means and standard deviations were determined for the mutation assay.

b. Evaluation criteria:

1) Assay validity: The assay was considered valid if the following criteria were met: (1) the spontaneous revertants of each strain fell within the reporting laboratory's acceptable range, and (2) all positive controls caused a "significant" increase in revertants per plate compared to the respective solvent control.

2) Positive response: The test material was considered positive if it caused a ≥2-fold increase in mean revertant colonies of strains TA98 or TA100 or if it caused a ≥3-fold increase in mean revertant colonies of strains TA1535, TA1537, or TA1538 over a minimum of three doses.

C. REPORTED RESULTS:

1. Preliminary Assay: Ten doses ranging from 10 to 10,000 µg/plate +/-S9 were assayed for cytotoxic effects on strain TA100.

Moderate to slight compound precipitation was observed on plates containing the three highest test doses (3,330, 6,670, and 10,000 µg/plate +/-S9). Without S9 activation, no colonies were seen at levels ≥6670 µg/mL. At 3330 µg/plate -S9, there was a slight reduction in the

background lawn of growth, but revertant colonies of S. typhimurium TA100 were not appreciably lower than the solvent control count. Our reviewers noted, however, that the background counts for TA100 (47 -S9 and 48 +S9) were extremely low (i.e., ≈50% lower than expected). In the presence of S9 activation, the high dose was completely cytotoxic. Lower than solvent control mutant colony counts were seen at the majority of S9-activated doses ≤6600 µg/mL, but the reductions were not clearly indicative of cytotoxicity. Based on these findings, six nonactivated doses ranging from 50 to 6,500 µg/plate and six S9-activated doses ranging from 100 to 10,000 µg/plate were selected for further evaluation.

2. Mutation Assays: Results from the first trial are presented in Table 1. As shown, concentrations higher than 1,000 µg/plate -/-S9 (2,500 and 6,500 µg plate -S9 and 2,500, 5,000, and 10,000 µg/plate -S9) precipitated. A slight reduction of the background lawn of growth was reported for all strains at the highest dose +/-S9. At the highest nonactivated dose (6500 µg/plate), revertant colony counts for all tester strains except TA1535 were reduced as compared with the appropriate solvent control values. With the exception of strain TA98, reduced revertant colony counts were seen at the highest S9-activated level (10,000 µg/plate). Approximately 60% lower than control mutant colonies of strain TA1537 were also noted at 2500 µg/plate -S9 and 5000 µg plate -S9. Below these levels, there was no cytotoxicity or mutagenicity.

Although there were minor differences in relative cytotoxicity, results from the confirmatory assay were generally in good agreement with the initial findings that the high dose of SAN 582H +/-S9 was cytotoxic in the majority of strains, but no evidence of a mutagenic effect was seen at any dose with or without S9 activation (Table 2).

Data presented in Table 2 also contain representative results from the repeat trial conducted with strain TA98. This portion of the confirmatory assay was repeated because higher than expected spontaneous reversion frequencies were seen for strain TA98. In general, these results compared favorably with the earlier findings and also indicate that SAN 582H was not mutagenic.

Salmonella

TABLE 1. Representative Results of the Initial Salmonella typhimurium Mutagenicity Assay with SAN 582H

Substance	S9 Activation	Dose (µg/plate)	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethylsulfoxide	-	--	8 ± 5	6 ± 2	4 ± 1	15 ± 2	106 ± 13
	+	--	8 ± 5	8 ± 6	14 ± 6	25 ± 7	130 ± 13
Positive Control							
Sodium azide	-	2.0	554 ± 11	--	--	--	596 ± 5
2-Nitrofluorene	-	1.0	--	--	218 ± 23	132 ± 13	--
ICR-191	-	2.0	--	95 ± 23	--	--	--
2-Anthramine	+	2.5	129 ± 20	134 ± 6	1072 ± 45	1017 ± 60	897 ± 22
Test Material							
SAN 582H	-	1000[b]	9 ± 3	4 ± 1	3 ± 1	10 ± 5	95 ± 19
	-	5000[b]	7 ± 1	2 ± 1	0 ± 1	9 ± 4	113 ± 12
	+	1000[b]	8 ± 3	8 ± 3	20 ± 3	25 ± 2	133 ± 5
	+	5000	9 ± 2	3 ± 2	14 ± 2	30 ± 6	113 ± 13
	+	10,000[c]	6 ± 2	4 ± 3	4 ± 3	23 ± 10	118 ± 1-

[a]Means and standard deviations of counts from triplicate plates.

[b]Highest nonprecipitating dose; results for remaining concentrations (50, 100, 500, and 2500 µg/plate -S9 and 100, 500, and 2500 µg/plate +S9) did not suggest a mutagenic response.

[c]Highest assayed dose; a slight reduction in the background lawn of growth was reported for all strains at this level both with and without S9 activation.

TABLE 2. Representative Results of the Confirmatory Salmonella typhimurium Mutagenicity Assay with SAN 582-

Substance	S9 Activation	Dose (µg/plate)	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA1538	TA98[b]	TA100
Solvent Control							
	-	--	13 ± 2	5 ± 3	6 ± 3	14 ± 5	94 ± 13
Dimethylsulfoxide	+	--	12 ± 2	7 ± 2	14 ± 4	24 ± 5	142 ± 12
Positive Control							
Sodium azide	-	2.0	543 ± 26	--	--	--	622 ± 41
2-Nitrofluorene	-	1.0	--	--	227 ± 21	110 ± 11	--
ICR-191	-	2.0	--	160 ± 10	--	--	--
2-Anthramine	+	2.5	209 ± 49	161 ± 10	1196 ± 11[c]	1010 ± 196	1126 ± 32
Test Material							
SAN 582-	-	1000[c]	9 ± 1	4 ± 1	5 ± 1	12 ± 7	86 ± 14
	-	6500[d]	3 ± 1	0 ± 1	0 ± 1	14 ± 3	0 ± 0
	+	1000[c]	14 ± 4	6 ± 1	12 ± 3	35 ± 5	135 ± 7
	+	5000	11 ± 4	5 ± 4	10 ± 2	29 ± 3	57 ± 18
	+	10,000[d]	7 ± 1	1 ± 1	3 ± 3	24 ± 8	0 ± 0

[a]Means and standard deviations of counts from triplicate plates.

[b]The assay was repeated with strain TA98 because a high spontaneous reversion was seen. Presented results are from the repeat assay with this strain.

[c]Highest nonprecipitating dose; results for remaining concentrations (50, 100, 500, and 2500 µg/plate -S9 and 100, 500, and 2500 µg/plate +S9) did not suggest a mutagenic response.

[d]Highest assayed dose; a slight reduction in the background lawn of growth was reported for all strains at this level both with and without S9 activation.

In contrast to the negative test material results, all strains responded to the appropriate nonactivated or S9-activated positive control in both the initial and confirmatory analysis.

Based on the overall findings, the study authors concluded that SAN 582H was not mutagenic in this test system.

D. REVIEWER'S DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and that the study authors interpreted the data correctly. In the presence or absence of S9 activation, SAN 582H was assayed over an appropriate range of test material concentrations. These included insoluble levels and a high dose that was cytotoxic in the majority of strains, with no indication of a mutagenic effect in a well-controlled study.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? YES . (A quality assurance statement was signed and dated June 8, 1989.)

F. ~~CBI APPENDIX~~: ~~Appendix A~~, Materials and Methods, CBI pp. ~~12-19~~.

APPENDIX A

Materials and Methods
CBI pp. 12-19

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EPA No.: 68D80056
DYNAMAC No.: 337-H
TASK No.: 3-37H
January 4, 1991

DATA EVALUATION RECORD

SAN 582H

Mutagenicity--Mammalian Cells in Culture Cytogenetic Assay in Chinese Hamster Ovary (CHO) Cells

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APPROVED BY:

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

Signature: Robert J. Weir

Date: Jan. 3, 1991

008285

Guideline Series 84: MUTAGENICITY

EPA No.: 68D80056
DYNAMAC No.: 337-H
TASK No.: 3-37H
January 4, 1991

DATA EVALUATION RECORD

SAN 582H

Mutagenicity--Mammalian Cells in Culture Cytogenetic Assay
in Chinese Hamster Ovary (CHO) Cells

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

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Toxicology Branch II
(H-7509C)

Signature: James Rowe
Date: 2/12/91

IN VITRO MAMMALIAN CYTOGENETICS

DATA EVALUATION RECORD

Tox. Chem. No.:
EPA File Symbol:

CHEMICAL: SAN 582H.

STUDY TYPE: Mammalian cells in culture cytogenetic assay in Chinese hamster ovary (CHO) cells.

ACCESSION OR MRID NUMBER: 415965-43.

SYNONYM/CAS NUMBER: None listed.

SPONSOR: Sandoz Crop Protection Corp., Des Plaines, IL.

TESTING FACILITY: Hazleton Biotechnologies Corp., Veenendaal, The Netherlands.

TITLE OF REPORT: SAN 582H *in vitro* Chromosome Aberration Assay Using Chinese Hamster Ovary (CHO) Cells.

AUTHOR: Taalman, R.D.F.M.

STUDY NUMBER: E-9428.

REPORT ISSUED: December 19, 1985.

CONCLUSIONS - Executive Summary: SAN 582H was evaluated for the potential to induce chromosome aberrations in Chinese hamster ovary (CHO) cells over a nonactivated dose range of 10 to 150 µg/mL and an S9-activated dose range of 150 to 500 µg/mL. Owing to severe cell-cycle delay, the nonactivated assay was conducted with a 20-hour cell harvest; no delay was seen with S9 activation; hence, a normal 10-hour harvest was performed. Cytotoxicity was apparent at nonactivated doses of 125 and 150 µg/mL and at S9-activated levels of 400 and 500 µg/mL.

Although the study author stated that SAN 582H was negative in this assay, we assess that no definitive conclusions can be reached. There is, however, sufficient evidence or suspected clastogenic activity to classify SAN 582H as a presumptive positive. We base this assessment on the increased incidence of chromatid-type aberrations observed both with and without S9 activation and the significant effects uncovered when our reviewers reevaluated the data using Fisher's Exact test (see Section C, Reported Results). Further, the unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes conducted with SAN 582H provided evidence of confirmed genotoxicity (see Data Evaluation Record 337-I), which supports our concern that the clastogenic potential of the test material was not fully evaluated.

Since unequivocal dose-related UDS activity was seen only when a narrow range of noncytotoxic levels of SAN 582H was examined in a confirmatory test, we assess that a similar approach should be employed in a repeat cytogenetic assay.

In addition to the issues raised above, there were no analytical data to support actual concentrations used in the study. Based on the above considerations, we conclude that a definitive result was not obtained; therefore, the study does not satisfy Guideline requirements for Category II, Structural Chromosome Aberrations.

Study Classification: The study is unacceptable and should be repeated with a range of test material doses that extends well below the cytotoxic dose. It should also be determined whether a narrow range of activity, similar to that reported in the UDS study, exists. Additionally, test material characterization (i.e., purity, stability, and storage condition) should be reported.

MAMMALIAN CELLS IN CULTURE GENE MUTATION

A. MATERIALS:

1. Test Material:

Name:	SAN 582H Technical
Description:	Brown liquid
Batch No.:	8505
Purity:	Not reported (Note: Batch No. 8605 was listed as 91.4% pure; see Data Evaluation Record 337-I.)
Contaminants:	Not listed
Solvent used:	Dimethylsulfoxide (DMSO)
Other comments:	Test material stability and storage conditions were not reported. SAN 582H was reported to be soluble in DMSO at 1 g/mL. Solutions of the test material were prepared in DMSO immediately prior to use.

2. Control Materials:
Negative: McCoy's 5a medium supplemented with 10% fetal calf serum, glutamine, and antibiotics.

Solvent/final concentration: DMSO/1%.

Positive: Nonactivation (concentrations, solvent): Mitomycin C (MMC) was prepared in distilled water at final concentrations of 0.005 and 0.01 µg/mL (cytotoxicity assay) and 0.5 and 1.0 µg/mL (cytogenetic assay).

Activation (concentrations, solvent): Cyclophosphamide (CP) was prepared in distilled water at final concentrations of 15 and 20 µg/mL (cytotoxicity assay) and 25 and 50 µg/mL (cytogenetic assay).

3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The S9 fraction was purchased from Litton Biological Products, Inc.

The composition of the S9 mix per mL of growth medium was as follows:

NADP	1.5 mg
Isocitric acid	2.7 mg
S-9	15 µL

4. Test Compound Concentrations Used:

   a. Preliminary cytotoxicity assay: Ten doses, ranging from 0.17 to 5000 µg/mL, separated by half-log dilutions, were evaluated with or without S9 activation.

   b. Cytogenetic assay:

      1. Nonactivated conditions: A dose range of 5 to 150 µg/mL was assayed with a delayed-cell harvest (20 hr).

      2. S9-Activated conditions: A dose range of 25 to 500 µg/mL was assayed with a normal-cell harvest (10 hr)

      Note: The intermediate doses evaluated under S9-activated conditions could not be determined by our reviewers because of the poor quality of the reproduced results.

5. Test Cells: The Chinese hamster ovary (CHO-WB1) cells used in this assay were originally obtained from Dr. Sheldon Wolff, University of California, San Francisco, CA. Prior to use, the CHO cells were grown 24 hours in McCoy's 5a medium.

   Properly maintained: Yes.

   Cell line or strain periodically checked for Mycoplasma contamination? Not reported.

   Cell line or strain periodically checked for karyotype stability? Not reported.

B. TEST PERFORMANCE:

1. Cell Treatments:

a. Cells exposed to test compound for:
17 hours (nonactivated) 2 hours (activated)

b. Cells exposed to positive controls for:
17 hours (nonactivated) 2 hours (activated)

c. Cells exposed to negative and/or solvent controls for:
17 hours (nonactivated) 2 hours (activated)

2. Protocol:

a. Preliminary assay: Prepared cultures, seeded at 0.75×10^5 cells/flask, were exposed with or without S9 activation to half-log dilutions of the test material (0.17 to 5000 µg/mL), the negative control (culture medium), solvent control (DMSO), or the positive controls (MMC -S9, CP -S9 +S9).

In the nonactivated system, cells were exposed for 2.5 hours to the test material; BrdU (10 µM) was added to the cultures, and incubation was continued for 20.75 hours. Cell monolayers were washed, refed fresh complete medium containing BrdU (10 µM), and reincubated in the presence of 0.1 µg/mL colcemid for 3 hours. In the S9-activated system, cultures were exposed for 2.25 hours without FCS. After exposure, cells were washed twice, refed complete medium containing BrdU (10 µM), and reincubated for 21.5 hours. Colcemid (0.1 µg/mL) was added, and cultures were incubated for an additional 3 hours.

After incubation, monolayers were visually examined for confluency; metaphase cells were collected by mitotic shake-off. Cells were swollen in a hypotonic 0.075-M solution of potassium chloride and washed three times in fixative (methanol:acetic acid, 3:1), and slides were prepared. Estimation of cell-cycle delay was accomplished by staining the cells using the modified fluorescent-plus-Giemsa techniques of Perry and Wolff[1]

[1]Perry, P. and Wolff, S. New Giemsa method for the differential staining of sister chromatids. Nature (1974) 251:156-158.

and Goto et al.[2] Cultures were examined for the percentage of first division (M_1), between first and second divisions (M_{1+}), and beyond second division ($>M_2$) metaphase cells; the number of cells scored was not specified. Based on the results, doses and harvest times were selected for the cytogenetic assay.

b. Cytogenetic assay:

1. Treatment: Prepared cultures (in duplicate), seeded at 1.5×10^6 cells, were exposed to the selected test material doses, the negative control (culture medium), solvent control (DMSO), or the positive controls (MMC -S9, CP +S9).

   In the nonactivated system, cells were dosed for 17 hours. Cultures were washed, refed medium containing colcemid, and reincubated for approximately 3 hours. Under S9-activated conditions, cells were exposed for 2 hours, washed, refed culture medium, and incubated for 8 hours. Colcemid was added 3 hours before the cultures were harvested.

   Metaphase cells were collected and fixed. Slides were stained with 5% Giemsa.

2. Metaphase analysis: One hundred cells per culture were scored for chromosome aberrations. Only 25 to 50 cells were scored from one of each positive control dose level. Chromatid and isochromatid gaps were counted, but not included, in the final analysis; these data were, however, not presented in the report.

3. Statistical methods: The data were compared to the pooled negative (culture medium) and solvent controls at $p \leq 0.05$ by the Chi-square test.

4. Evaluation criteria: No criteria to establish assay validity or a positive response were presented. The biological significance of the results was evaluated relative to the overall chromosome aberration frequencies, percentage of

[2]Goto, K., Maeda, S., Kano, Y., and Sugiyama, T. Factors involved in differential Giemsa-staining of sister chromatids. Chromosoma (1978) 66:351-359.

cells with aberrations, percentage of cells with ≥1 aberration, dose-response, and the type of aberrations observed.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay:

The cytotoxicity assay was conducted with test doses ranging from 0.17 to 5000 µg/mL, separated by half-log dilutions, in the presence and absence of S9 activation.

Two range-finding assays were initiated; the first test was aborted because of fungal contamination, and the results from the second assay were used to set doses for the cytogenetic evaluation.

a. Without S9 activation: Compound precipitation was reported at the two highest doses (1670 and 5000 µg/mL), and no cells survived dosing at levels ≥166.7 µg/mL. Marked reductions in mitotic cells and severe depression of cell-cycle kinetics were observed at 50 µg/mL (Table 1). Below this level, there was no definitive cell cycle delay. Based on these findings, the nonactivated cytogenetic assay was performed with a dose range of 5 to 150 µg/mL and a delayed cell harvest (20 hours).

b. With S9 activation: Compound precipitation was reported at 1670 and 5000 µg/mL, and complete cytotoxicity occurred at doses ≥500 µg/mL. Below 500 µg/mL, there was no appreciable effect on cell cycling (Table 1); accordingly, a dose range of 25 to 500 µg/mL with a normal 10-hour cell harvest was selected for the S9-activated cytogenetic assay.

2. Cytogenetic Assay:

a. Nonactivated results: Severe cytotoxicity was reported at the two highest nonactivated levels of SAN 582H (125 and 150 µg/mL). Accordingly, metaphases harvested from cultures exposed to 10, 25, 50, 75, and 100 µg/mL -S9 were scored for chromosome aberrations. Although there were no significant increases (Chi-square test) in the percentage of cells with aberrations, this value was higher than the pooled negative control at 50, 75, and 100 µg/mL (Table 2). Our reviewers also noted a relatively high incidence of chromatid breaks (14) at the

MAMMALIAN CELLS IN CULTURE GENE MUTATION

TABLE 1. Results from the Preliminary Test for Delay of Cell-Cycle Progression with SAN [illegible]

Substance	Dose/mL	S9 Activation	% Cells[a] M_1	% Cells[a] M_{1+}	% Cells[a] $>M_2$
Negative Control					
Culture medium	--	-	11	0	89
	--	+	0	0	100
Solvent Control					
Dimethylsulfoxide	1%	-	24	0	76
	1%	+	10	0	90
Positive Control					
Mitomycin C	0.005 µg	-	26	0	74
Cyclophosphamide	1[illegible] µg	+	52	0	48
Test Material					
SAN 582H	16.7 µg[b]	-	37	0	63
	50.0 µg[b]	-	95	0	5
	166.7 µg[b,c]	+	22	0	78

[a] Percent cells in first division (M_1), between first and second division (M_{1+}), or beyond second division ($>M_2$).

[b] Results for 5.0 and 1.7 µg/mL showed no definitive evidence of cell-cycle delay; lower levels were not scored.

[c] Higher levels (166.7, 500, 1670, and 5000 µg/mL -S9 and 500, 1670, and 5000 µg/mL +S9) were completely cytotoxic.

[d] Results for 50, 16.7, and 5.0 µg/mL did not indicate adverse effects on cell-cycle kinetics; lower levels were not scored.

Note: Compound precipitation was reported for the two highest assayed doses (1670 and 5000 µg/mL +/-S9).

TABLE 2. Representative Results of the CHO Cell *in vitro* Cytogenetic Assay with SAN 582H Following a 20-Hour Nonactivated Harvest

Substance	Dose/mL	No. of Cells Scored	Total Aberrations	Aberrations per Cell	p Value[a]	% Cells with Aberrations	p Value[a]	% Cells with >1 Abberation	Biologically Significant Aberrations (No./Type)
Pooled Negative Control									
McCoy's 5a medium	-	200	2	0.010	--	1.0	--	0.0	2TB
Dimethylsulfoxide									
Positive Control									
Mitomycin C	0.5 µg	25	32	1.280	ND	80.0[b]	ND	28.0	21TB; 5TR; 1QR; 1SB; 4AF
Test Material									
SAN 582H	50 µg[c]	200	10	0.050	0.0179*	2.0	0.3426	1.0	3TB; 1D; 6DM
	75 µg	200	6	0.030	0.1420	2.5	0.2245	0.5	2TB; 2D; 2R
	100 µg[d]	170	15	0.090	0.0003**	4.1 (4.6)[e]	0.0539 (0.0297)*	1.2	14TB; 1SB

[a]Fisher's Exact test: * - Significantly higher than the pooled negative control value ($p < 0.05$).
** - Significantly higher than the pooled negative control value ($p < 0.01$).

[b]Significantly higher than the pooled negative control value ($p < 0.05$) as determined by the Chi-square test.

[c]Results for lower doses (25, and 10 µg/mL) showed no significant effects.

[d]Higher levels (125 and 150 µg/mL) were severely cytotoxic and, therefore, not scored.

[e]Recalculation of the presented data revealed that 4.6% of the cells had aberrations.

Abbreviations used:

TB - Chromatid break
TR - Triradial
QR - Quadriradial
SB - Chromosome break
AF - Acentric fragment
D - Dicentric
DM - Double minute
R - Ring
ND - Not done

high dose. Similarly, four complex aberrations (2 dicentrics and 2 rings) were seen at 75 μg/mL, and one complex (dicentric) and nine simple aberrations (3 chromatid breaks and 6 double minutes) were scored at the 50-μg/mL dose level. Our reviewers, therefore, reevaluated the data using Fisher's Exact test, which is generally considered to be the appropriate method for statistical analysis of cytogenetic assay results.

As shown in Table 2, aberrations per cell were significantly higher at 50 μg/mL ($p < 0.05$) and 100 μg/mL ($p < 0.001$). In addition, our reviewers noted that the combined percentage of cells with aberrations (i.e., total number of cells with aberrations divided by total number of cells scored and multiplied by 100) presented by the author for the highest nonactivated dose (4.1%) was incorrect. Recalculation of the available data indicated that the actual value was 4.6%. Both values were analyzed by Fisher's Exact test; it was found that 4.6% cells with aberrations was significant ($p < 0.05$) and the 4.1% value was borderline nonsignificant.

Based on the reevaluation of the data, our reviewers concluded that there is adequate evidence of suspect clastogenic activity induced by nonactivated SAN 582H.

b. S9-activated results: In the presence of S9 activation, cytotoxicity was reported for the two highest doses (400 and 500 μg/mL). Metaphases were scored from cultures exposed to four doses (150 to 400 μg/mL); the actual concentrations of the two intermediate doses could not be determined owing to the poor quality of the presented results. Nonsignificant (Fisher's Exact and Chi-square tests) increases in the percentage of cells with aberrations were observed for all treatment groups (Table 3). Our reviewers again noted increased frequencies of chromatid breaks, and a significant ($p < 0.05$) increase in the number of aberrations per cell for the 400-μg/mL dose group.

Based on the overall findings, the study author concluded that SAN 582H is not clastogenic in this test system.

D. Reviewers' Discussion/Conclusions: We disagree with the study author's conclusion that SAN 582H is not clastogenic in this test system. Although the results are not conclusive, there is sufficient evidence to classify SAN 582H as a presumptive positive. We based this assessment on the increased incidence of chromatid-type aberrations both with and without S9 activation, and the significant effects uncovered when the data were analyzed by the Fisher's Exact test. In addition, the findings from the unscheduled DNA synthesis assay in primary rat

TABLE 3. Representative Results of the CHO Cell in vitro Cytogenetic Assay with SAN 582H Following a 10-Hour S9-Activated Harvest

Substance	Dose/mL	No. of Cells Scored	Total Aberrations	No. of Aberrations per Cell	p Value[a]	% Cells with Aberrations	p Value[a]	% Cells with >1 Aberration	Biologically Significant Aberrations (No./Type)
Pooled Negative Control									
Dimethylsulfoxide	--	200	3	0.015	--	1.5	--	0.0	3TB
Positive Control									
Cyclophosphamide	50.0 µg	25	13	0.520	ND	36.0[b]	ND	12.0	7TB; 2TR; 3QR; 1ID
Test Material									
SAN 582H	150 µg	200	4	0.020	0.5000	2.0	0.5000	0.0	4TB
	? µg[c]	200	8	0.040	0.1100	4.0	0.1100	0.0	8TB
	? µg[c]	200	6	0.030	0.2514	2.5	0.3619	0.5	6TB
	400 µg[d]	200	11	0.055	0.0266*	4.5	0.0700	1.0	11TB

[a]Fisher's Exact test: *Significantly higher than the pooled negative control value ($p < 0.05$).

[b]Significantly higher than the pooled negative control values ($p < 0.05$) as determined by the Chi-square test.

[c]The actual concentration of the two intermediate doses could not be determined due to the poor quality of the reported data.

[d]Highest assayed level (500 µg/mL) was severely cytotoxic and, therefore, not scored.

Abbreviations used:

TB - Chromatid break
TR - Triradial
QR - Quadriradial
ID - Interstitial deletion
ND - Not done

hepatocytes conducted with SAN 582H (see Data Evaluation Record 337-I) clearly demonstrated that the test material was genotoxic and that UDS activity occurred over a narrow range of test doses that were well below the cytotoxic level. We assess, therefore, that a definitive conclusion as to whether SAN 582H is a clastogen can be reached only by repeating the cytogenetic assay with a range of test doses that extends well below the cytotoxic dose. It should also be determined whether a narrow range of activity, similar to that reported in the UDS study, exists.

E. Quality Assurance: Was test performed under GLPs? Yes. (A quality assurance statement was signed but not dated.)

F. CBI Appendix: Appendix A, Protocol CBI pp. 13-21.

APPENDIX A

Protocol
(CBI pp. 13-21)

DIMETHENAMID RIN 2014-93

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EPA No.: 68D80056
DYNAMAC No.: 337-I
TASK No.: 3-37I
January 7, 1991

DATA EVALUATION RECORD

SAN 582H

Mutagenicity--Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes

APPROVED BY:

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

Signature: Robert J Weir

Date: 1-4-91

008285

Guideline Series 84: Mutagenicity
EPA No.: 68D80056
DYNAMAC No.: 337-I
TASK No.: 3-37I
January 7, 1991

DATA EVALUATION RECORD

SAN 582H

Mutagenicity--Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
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DATA EVALUATION RECORD

Tox. Chem. No.:
EPA File Symbol:

CHEMICAL: SAN 582H.

STUDY TYPE: Unscheduled DNA synthesis assay in primary rat hepatocytes.

MRID NUMBER: 415965-44.

SYNONYMS/CAS NUMBER. None listed.

SPONSOR: Sandoz Crop Protection Corp., Des Plaines, IL.

TESTING FACILITY: Hazleton Laboratories America, Inc., Kensington, MD.

TITLE OF REPORT: Mutagenicity Test on SAN 582H in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay.

AUTHOR: M.A. Cifone.

STUDY NUMBER: 10767-0-447.

REPORT ISSUED: November 7, 1989.

CONCLUSION(S) - Executive Summary: Eight doses of SAN 582H ranging from 0.025 to 10 µg/mL were evaluated in the unscheduled DNA synthesis (UDS) assay. Higher concentrations were not scored for UDS owing to severe cytotoxicity. Results indicated that doses ranging from 0.1 to 10.0 µg/mL did not induce a genotoxic response in primary rat hepatocytes. However, increased net nuclear grain counts and increased percentages of nuclei with ≥6 grains were seen at the two lowest assayed levels (0.025 and 0.05 µg/mL). The assay, therefore, was repeated using 15 doses ranging from 0.01 to

50 µg/mL. Owing to high cytotoxicity at >10.0 µg/mL, the levels scored for UDS were 0.1, 0.25, 1.0, 2.5, 5.0, and 10.0 µg/mL of the test material. Survival for the scored treatment groups ranged from 76.3% at the high dose to 98.1% at the low dose. Slight increases in both parameters used to measure UDS activity were seen at 2.5, 5.0, and 10.0 µg/mL. However, a clear dose-related increase in net nuclear grains and in the percentage of nuclei with ≥6 grains, which exceeded the reporting laboratory criteria for a positive response, were observed at 0.1, 0.25, and 1.0 µg/mL. It was noteworthy that the increase in nuclear grains at 1.0 µg/mL (21.05 net nuclear grains) was higher than the positive control (19.01 net nuclear grains for 0.1 µg/mL 2-acetylaminofluorene). Additionally, the increased percentage of nuclei with ≥6 grains for 1.0 µg/mL SAN 582H (92.7%) was only slightly lower than the positive control (94.7%). Although the decline in UDS activity at concentrations higher than 1.0 µg/mL would be consistent with a cytotoxic effect, neither the average cytoplasmic grain counts nor the percent cell survival at levels >1.0 µg/mL indicated cytotoxicity. We conclude, therefore, that SAN 582H was genotoxic in this test system and that UDS activity occurred at doses well below the cytotoxic level. Since the results are unequivocally positive and positive responses are rarely reported for this assay, we further assess that this finding is highly significant and could have implications for other genetic toxicology assays performed with SAN 582H. Based on the evidence of a clear positive response, we conclude that the study fulfills Guideline requirements for Category III, Other Mutagenic Mechanisms.

Study Classification: The study is acceptable; SAN 582H is a confirmed positive in the primary rat hepatocyte UDS assay.

RECOMMENDATIONS: Since a dose-related positive response was demonstrated for this test material at concentrations well below cytotoxic levels, it is recommended that all other genetic toxicology assays conducted with SAN 582H be carefully reviewed with these findings in mind.

A. MATERIALS:

1. Test Material:

Name:	SAN 582H, technical
Description:	Dark brown liquid
Lot No.:	3605
Purity:	91.4%
Contaminants:	Not listed
Solvent used:	Dimethylsulfoxide (DMSO)

Other comments: The test material was soluble in DMSO at ≈104 mg/mL. Solutions of the test material were freshly prepared on the day of use.

2. Indicator Cells: Primary rat hepatocytes were obtained by the in situ perfusion of the livers of adult male Fischer 344 rats weighing 226.6 to 260.5 g and purchased from Harlan Spraque-Dawley, Inc.

3. Control Substances: DMSO at a final concentration of 1% was used as the solvent control, and 2-acetylaminofluorene (2-AAF) at 0.1 µg/mL was used as the positive control.

4. Medium: WMEI: Williams' Medium E supplemented with 2 mM L-glutamine and antibiotics; WME+: As above with 10% fetal calf serum.

B. STUDY DESIGN:

1. Cell Preparation:

a. Perfusion technique: Livers were perfused with Hanks' balanced salts containing 0.5 mM EGTA and HEPES buffer, pH 7.2, for 4 minutes and with WMEI containing 50 to 100 units/mL collagenase for 10 minutes. Livers were excised, placed in culture dishes containing WMEI and collagenase, and mechanically dispersed to release the hepatocytes.

b. Hepatocyte harvest/culture preparation: Recovered cells were centrifuged, resuspended in WME+, counted, and aliquoted (0.5×10^6 cells/3 mL WME+) onto plastic coverslips. The cultures were placed in a humidified, 37°C, 5% CO_2 incubator for a 1.6- to 1.8-hour attachment period. Unattached cells were removed; viable cells were refed with WMEI and established as monolayer cultures.

2. Dose Selection: Initially, 15 concentrations of the test material (0.1 to 500 µg/mL in Trial I and 0.01 to 50 µg/mL in Trial II) were assayed. When the viability estimate was obtained (20.7 to 21.2 hours after treatment initiation), at least six of these doses were chosen for analysis of nuclear labeling, starting with the highest dose that resulted in a sufficient number of survivors with intact morphologies and proceeding to successively lower doses.

3. UDS Assay:

a. Treatment: Five replicate monolayer cultures were exposed to the selected doses of the test material, negative control (DMSO), or positive control (2-AAF) for 18.3 to 18.9 hours in WMEI containing 5 µCi/mL [^{3}H]thymidine. Treated monolayers were washed twice with WMEI; two of the five replicates for each treatment group were used to determine cytotoxicity. These cultures were refed, reincubated, and monitored for cytotoxicity at 20.7 to 21.2 hours posttreatment by trypan blue exclusion.

b. UDS slide preparation: The remaining cultures were washed with media containing 1mM thymidine. Treated hepatocytes, attached to coverslips, were exposed to 1% sodium citrate for ≈10 minutes, fixed in acetic acid:ethanol (1:3), dried, and mounted.

c. Preparation of autoradiographs/grain development: Slides were coated with Kodak NTB2 emulsion, dried for 7 to 10 days at 4°C in light-tight desiccated boxes, developed in Kodak D-19, fixed, stained with Williams' modified hematoxylin and eosin, coded, and counted.

d. Grain counting: The nuclear grains of morphologically normal cells (50/coverslip) for each test dose and negative and positive controls were counted microscopically. Net nuclear grain counts were determined by subtracting the average cytoplasmic grain count of three nuclear-sized areas adjacent to each nucleus from the nuclear grain count of each cell.

4. Evaluation Criteria:

a. Assay validity: For the assay to be considered valid, the following criteria must be satisfied: (1) hepatocytes recovered from the perfusion step and monolayer cultures used for the assay must show ≥70% viability; (2) the negative or solvent control should have net nuclear grain counts of -5.0 to 1.0, and ≤10% of the cells should contain ≥6 grains/nucleus; (3) the positive control must demonstrate the sensitivity of the test system to detect UDS; (4) data must be obtained from at least two replicate cultures/dose; and (5) the highest dose must show cytotoxicity, the limit of solubility, or reach the maximum recommended dose for this assay (5 mg/mL).

b. Positive response: The assay was considered positive if: (1) the increase in the mean net nuclear grain count was ≥6 grains/nucleus over the negative control value after subtraction of the concurrent negative control value, and/or (2) the percentage of nuclei with ≥6 grains exceeded 10% of the negative control population after subtraction of the concurrent negative control value.

C. REPORTED RESULTS:

Two trials of the UDS assay were performed with 15 doses ranging from 0.1 to 500 µg/mL (Trial 1) and 0.01 to 50 µg/mL (Trial 2). The report indicated that concentrations ≥250 µg/mL were insoluble in culture medium. The results of the individual trials were as follows:

1. Trial 1: The study author stated that levels of SAN 582H >10 µg/mL were severely cytotoxic. At 10 µg/mL, 84.2% of the cells survived treatment; therefore, hepatocytes exposed to 10, 5, 2.5, 1.0, 0.5, 0.1, 0.05, and 0.025 µg/mL were scored for UDS activity. The net nuclear grain count and the percent of nuclei with ≥6 grains was not increased compared to the concurrent solvent control at doses ranging from 0.1 to 10 µg/mL SAN 582H. However, as shown in Table 1, increased net nuclear grain counts and increased percentages of nuclei with ≥6 grains were scored at the two lowest assayed levels (0.05 and 0.025 µg/mL). The increase in the percentage of nuclei with ≥6 grains at 0.025 µg/mL (33%) exceeded the reporting laboratories positive response criterion for this parameter (≥19.3% higher than concurrent solvent control). Based on these findings, a repeat assay was performed.

2. Trial 2: For the repeat trial, a narrower range of 15 test material concentrations (0.01 to 50 µg/mL) were assayed. The authors stated that levels >10 µg/mL were severely cytotoxic; accordingly doses ranging from 0.1 to 10 µg/mL were scored. As shown in Table 2, percent survival ranged from 76.3% at 10 µg/mL to 98.1% at 0.1 µg/mL. All scored doses induced an increase in net nuclear grain counts and the percentage of cells with ≥6 grains/nucleus. Increased net nuclear grain counts, which exceeded the reporting laboratory's criterion for a positive response (i.e., 6.3 nuclear grains) were counted at 0.25 and 1.00 µg/mL. The

TABLE 1. Representative Results of the Unscheduled DNA Synthesis Rat Hepatocyte Assay with SAN 582H: Trial I

Treatment	Dose/mL	Cells Scored	Percent Survival (21.2 hours post-exposure)	Average Cytoplasmic Grain Count[a]	Average Net Nuclear Grain Count[a]	Average Percent Nuclei with ≥ 6 Grains[a]
Solvent Control						
Dimethylsulfoxide	1%	150	100.0	11.19	0.47	9.3
Positive Control						
2-Acetylamino-fluorene	0.1 μg	150	65.8	10.81	13.32[b]	39.3[b]
Test Material						
SAN 582H	0.025 μg	150	77.6	11.03	2.99	33.7
	0.05 μg	150	79.3	9.10	1.58	16.0
	0.10 μg	150	87.5	12.09	-1.19	2.7
	10.00 μg[c]	150	84.2	13.15	-2.00	3.3

[a]Average value for triplicate coverslips, except the 10.0- μg/mL dose group; one slide could not be analyzed.

[b]Fulfills reporting laboratory's criteria for a positive effect (i.e., >6.47 net nuclear grains and/or ≥19.3% of nuclei with ≥6 grains).

[c]Highest concentrations scored for UDS activity; higher levels were severely cytotoxic. Results for intermediate doses (5, 2.5, 1.0, and 0.5 μg/mL) were negative for UDS activity.

TABLE 2. Results of the Unscheduled DNA Synthesis Rat Hepatocyte Assay with SAN 582H: Trial II

Treatment	Dose/mL	Cells Scored	Percent Survival (20.7 hours post-exposure)	Average Cytoplasmic Grain Count[a]	Average Net Nuclear Grain Count[a]	Average Percent Nuclei with ≥ 6 Grains[a]
Solvent Control						
Dimethylsulfoxide	1%	150	100.0	10.73	0.30	6.7
Positive Control						
2-Acetylamino-fluorene	0.1 µg	150	78.1	14.31	19.01[b]	94.7[b]
Test Material						
SAN 582H	0.10 µg	150	98.1	10.22	5.09	46.0[b]
	0.25 µg	150	90.2	9.61	8.74[b]	70.0[b]
	1.00 µg	150	86.1	13.31	21.05[b]	92.7[b]
	2.50 µg	150	93.0	11.74	2.59	22.7[b]
	5.00 µg	150	96.0	11.80	0.73	13.3
	10.00 µg	150	76.3	8.24	0.95	10.0

[a]Average value for triplicate coverslips.

[b]Fulfills reporting laboratory's criteria for a positive effect (i.e., >6.30 net nuclear grains and/or ≥16.7% of nuclei with ≥6 grains).

percentage of cells with ≥6 grains for the 2.5-, 1.0-, 0.25-, and 0.1-μg/mL dose groups also exceeded the minimum increase required to conclude a positive response. It was noted that the response induced by 1.0 μg/mL SAN 582H was equivalent to the response elicited by the positive control, 0.1 μg/mL 2-AAF. At higher concentrations of SAN 582H, UDS activity decreased; however, the values for 5.0 and 10.0 μg/mL were higher than the solvent control. Although the decline in UDS activity suggests that cytotoxicity occurred at levels >1.0 μg/mL, neither the average cytoplasmic grain counts nor percent survival indicated that a cytotoxic effect was responsible for the decline.

Based on the overall results, the study author concluded that SAN 582H was active in the rat primary hepatocyte UDS assay.

D. REVIEWERS' DISCUSSION/INTERPRETATION ~~OF STUDY~~ RESULTS:

We assess in agreement with the study author that SAN 582H was genotoxic in this test system. The initial evidence of UDS activity was confirmed in the second trial. We further assess that the demonstration of a dose-related effect at concentrations well below cytotoxic levels may have implications for other genetic toxicology assays performed with SAN 582H. Since our reviewers have rarely seen an unequivocal positive response in this test system, we consider this finding to be highly significant. It appears that the genotoxic response is not correlated with the cytotoxic effects of SAN 582H. The author stated that "the unusual toxicity curve appears to be a property of the test material and not due to problems with the study." This suggests that genotoxicity may occur only within a restricted dose range. We conclude, therefore, that all genetic toxicology assays performed with SAN 582H should be carefully reviewed with these considerations in mind.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement was signed and dated November 7, 1989.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 11-13.

APPENDIX A

Materials and Methods

CBI pp. 11-18

DIMETHENAMID RIN 2014-93

Page_____ is not included in this copy.

Pages 230 through 237 are not included.

The material not included contains the following type of information:

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008285

EPA No.: 68D80056
DYNAMAC No.: 337-J
TASK No.: 3-37J
January 30, 1991

DATA EVALUATION RECORD

SAN 582H

Metabolism in Rats

STUDY IDENTIFICATION: Vollin, S. SAN 582H metabolism in the rat. (Unpublished study No. 12726/89 performed by Sandoz, Ltd., Basle, Switzerland; dated November 13, 1989.) MRID No. 415965-45.

APPROVED BY:

Robert J. Weir, Ph.D.
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Signature: Robert J. Weir

Date: 1/30/91

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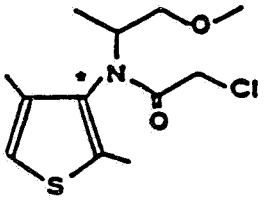
1. CHEMICAL: SAN 582H; 2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl) acetamide.

2. TEST MATERIAL: Unlabeled SAN 582H and SAN 582H labeled with ^{14}C in position 3 of the thiophene ring were used. The analytical-grade unlabeled test material was 99.8% pure, and [^{14}C]SAN 582H had a specific activity of 157 uCi/mg and a radiochemical purity of >99%. The structure and radiolabel position (*) of [^{14}C]SAN 582H are shown below:



3. STUDY/ACTION TYPE: Metabolism in rats.

4. STUDY IDENTIFICATION: Vollin, S. SAN 582H metabolism in the rat. (Unpublished study No. 12726/89 performed by Sandoz, Ltd., Basle, Switzerland; dated November 13, 1989.) MRID No. 415965-45.

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7. CONCLUSIONS:

A. Single low and single high oral doses of [^{14}C]SAN 582H and a single low oral dose of [^{14}C]SAN 582H following repeated low oral doses of unlabeled compound were readily absorbed and eliminated by male and female rats. Elimination of radioactivity was essentially complete within 3 days after dosing, and more than 90% of the ^{14}C dose was excreted in the urine and feces or urine, feces, and bile within 7 days. The total fraction of radioactivity eliminated was independent of sex and dose. However, females excreted more of the radioactive dose in the urine (47 to 63%) and less in the feces (26 to 48%) than males (about 30 and 62%, respectively). In addition, urinary levels of ^{14}C were much higher for high-dose rats (62 to 63% of the dose) than for all other groups (31 to 53%), indicating possible saturation of biliary excretion in the high-dose animals. Recovery of 75 to 82% of a single oral low dose of 10 mg [^{14}C]SAN 582H/kg in the bile demonstrated that the primary route of elimination of the parent compound and its metabolites is the liver.

SAN 582H was extensively metabolized by all groups within the first 3 days after administration of the ^{14}C-labeled test material. Less than 2.5% of the ^{14}C dose was recovered as unchanged parent compound, and 22 metabolites, 21 of which were found in both the urine and feces, were identified. The 22 metabolites plus unchanged SAN 582H accounted for approximately 22 to 39% of the orally administered radioactivity; no metabolite accounted for more than 10% of the ^{14}C dose, and most represented less than 2% (free and conjugated forms combined). An additional 18 to 30% of the orally administered radioactivity was excreted as "polar" and/or conjugated material that was not characterized further, and approximately 18 to 29% was extracted but not definable. Essentially all of the urinary radioactivity was extractable, but up to 12% of the ^{14}C dose remained bound to the feces.

On the basis of the metabolites identified in the urine and feces, the primary metabolic pathways for SAN 582H involve glutathione conjugation via displacement of the chlorine atom followed by (1) breakdown of glutathione to mercapturic acid or (2) hydrolysis of the thio bond of the mercaptan, which is then methylated and oxidized to the methylsulfoxide and methylsulfone. Additional major metabolic reactions include O-demethylation of the 2-methoxy-1-methylethyl moiety to form a 2-hydroxylated product and oxidation of the 2-methyl group on the thiophene ring to produce a hydroxymethyl. Dimerization, cyclization, and hydroxylation at the thiol also occur

following hydrolysis of the glutathione conjugate. Other minor pathways for SAN 582H involve direct biotransformation reactions such as reductive chlorination, oxidation of the sulfur on the thiophene ring to form a sulfoxide, oxidation of the 2- or 4-methyl group on the thiophene ring, and cyclization and hydroxylation of the thiol. Qualitatively, the metabolic pathways for SAN 582H appeared to be independent of sex and dose. Quantitatively, saturation of the glutathione conjugation pathway may have occurred at the higher dose.

B. Classification: (Core Supplementary) Unacceptable - bm 2/11/91

This study provides supplementary information on the metabolism of SAN 582H in male and female rats for all treatment regimens required by EPA (Guideline 85-1): single low, single high, and repeated low oral dosing. The study does not meet EPA requirements, however, because between 61 and 78% of the ^{14}C dose was not identified or was characterized only as polar material. Information on tissue ^{14}C distribution and retention also was not included in this report but was part of another study (see footnote 2 of this DER). The study could be upgraded through the inclusion of (1) tissue distribution/retention data and complete chromatographic results; (2) dose selection rationale; and (3) further identification of metabolites, or at least an explanation as to why more of the excreted material was not identified.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1) [^{14}C]SAN 582H (batch No. 1, synthesis No. RA683), labeled in position 3 of the thiophene ring, had a specific activity of 157 μCi/mg and a radiochemical purity of >99%. Unlabeled analytical-grade SAN 582H was 99.8% pure (analysis No. 20278).

2) Male and female Kfm:WIST rats (purchased from Madorin, Switzerland) were used. Information on body weights, acclimatization period, and feed/fasted state of the animals at the time of dosing was not given.

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[1]Only items appropriate to this DER have been included.

3) Information on the preparation of the dosing solution was not provided.

4) This report describes the isolation, identification, and quantification of the metabolites of [^{14}C]SAN 582H in the urine, feces, and bile of rats used in a previously conducted pharmacokinetics study.[2] Five experiments were conducted. Groups of six males and six females were assigned to one of the following: (1) the low-dose group, in which animals were given a single oral dose of 10 mg [^{14}C]SAN 582H/kg; (2) the high-dose group, in which rats received a single oral dose of 1000 mg [^{14}C]SAN 582H/kg; (3) the repeated-dose group, in which rats were given a single oral dose of 10 mg unlabeled SAN 582H/kg/day for 14 days followed by a single oral dose of 10 mg [^{14}C]SAN 582H/kg on day 15; or (4) the intravenous (iv)-dose group, in which animals received a single injection of 10 mg [^{14}C]SAN 582H/kg. A fifth group of six bile duct-cannulated rats (three/sex) were given a single oral dose of 10 mg [^{14}C]SAN 582H/kg.

Urine, feces, and bile were collected for 7 days (168 hours) after administration of the radiolabeled test material. Since excretion of ^{14}C by all routes was essentially complete within 72 hours after dosing, excreta collected between 0 and 72 hours post-dosing were pooled separately by sex for each experiment.

5) a. Aliquots of acidified (pH 2 to 3), pooled urine samples from all but the bile duct-cannulated animals were desalted on an Amberlite XAD-2 column. Before use, the XAD-2 resin was kept in methanol overnight, passed through a fritted glass filter, washed several times with deionized water, added to the column, and rinsed with dilute (0.1 N) HCl. After the acidified urine was added, the column was washed with dilute HCl and eluted with methanol; the washings and eluate were analyzed for ^{14}C content by liquid scintillation counting (LSC).

The methanolic eluate was concentrated, and the remaining aqueous solution was transferred to a separatory funnel, diluted with water, and

[2]Schweitzer, A. SAN 582H: Adsorption, distribution, and excretion in rats after single and multiple doses of ^{14}C SAN 582H. Unpublished study performed by Sandoz, Ltd., Basle, Switzerland, February 1988.

adjusted to pH 7 using dilute (1 N) NaOH. The transferred material was extracted twice with dichloromethane; the remaining aqueous phase was adjusted to pH 2 using 1 N HCl and extracted as before. Extracts were combined by pH, dried over anhydrous sodium sulfate, and concentrated. The concentrates were transferred to 10-mL flasks and diluted to volume. Aliquots of the dichloromethane extracts and the remaining aqueous phase were counted.

The aqueous phase was neutralized to pH 7 with NaOH, divided, and lyophilized. The powdered material was dissolved in sodium acetate buffer (0.1 M, pH 5.0). One reconstituted sample was incubated with ß-glucuronidase and arylsulfatase for 24 hours; the other sample was held under the same incubation conditions but was not treated with enzymes (control). After incubation, the samples were diluted with water and extracted with dichloromethane (three times at pH 7 and then three times at pH 2). The extracts were concentrated, and aliquots of the organic extracts and the aqueous phase were analyzed for ^{14}C content. The amount of enzyme-hydrolyzed metabolites was calculated from the difference in the radioactive content between the treated and control samples.

The aqueous phase remaining after dichloromethane extraction of the enzyme-hydrolyzed urine sample was treated with 3N NaOH and stirred for 72 hours at 100°C. The sample was then neutralized with 1N HCl, the precipitated material was filtered, and the remaining material was washed twice with water. The filtrate was extracted with dichloromethane at pH 7 and pH 2, and the extracts were assayed for radioactivity via LSC. Material remaining in the filter was air dried and weighed; aliquots were combusted and then counted.

Aliquots of the aqueous phases remaining after enzymatic or chemical hydrolysis were acetylated and methylated and analyzed for ^{14}C content.

Methods used to process the urine of bile duct-cannulated rats were not described.

b. Aqueous suspensions of homogenized feces from all rats were centrifuged; the supernatant was collected, and the precipitated material was resuspended in methanol and homogenized. The

homogenates were filtered, and the filter cake was extracted twice with methanol. The combined methanol extracts were concentrated, and the remaining aqueous material was added to the supernatant collected after centrifugation of the homogenized feces. Aliquots of the extracts were analyzed for ^{14}C content. Extracted feces were air dried and weighed; aliquots were combusted prior to counting.

The aqueous extracts were extracted with dichloromethane three times at pH 7 and three times at pH 2 as described for methanolic eluates of urine. Extracts were combined by pH, dried with anhydrous sodium sulfate, and concentrated. Aliquots of the dichloromethane extracts and of the remaining aqueous phase were assayed for radioactivity.

The extracted aqueous phase was treated with 3N NaOH, stirred for 72 hours (100°C), neutralized with 1N HCl, and processed as described for the aqueous fecal extracts (immediately preceding paragraph). Aliquots of the extracted air-dried feces were hydrolyzed under the same conditions. Before extraction, the neutralized samples were filtered, and the filter cakes were air dried, combusted, and analyzed for ^{14}C content by LSC.

c. Methods for the partitioning, enzymatic and chemical hydrolysis, and ^{14}C determination of bile samples were the same as for those of urine collected from all but the bile duct-cannulated animals, with the following exception: for partitioning, aliquots of bile samples were first diluted with water; samples then underwent two series of three extractions with dichloromethane, first at pH 7 and then at pH 2.

d. Appropriate measures were taken to determine counting efficiencies and to monitor color quenching during assaying of radioactivity.

5) Structural characterization of metabolites of SAN 582H was done using urine from high-dose rats. The metabolites in high-dose urine were separated via chromatographic methods [preparative thin-layer liquid chromatography (TLC) and high-performance liquid chromatography (HPLC)] and characterized by spectroscopic methods [nuclear magnetic resonance (NMR) and mass spectrometry (MS)]. Prior to these analyses, high-dose urine samples were pooled and extracted as described in

section (5)(a) of this DER. Methanolic extracts were concentrated, and the aqueous concentrate was extracted with dichloromethane at pH 7 and pH 2. The remaining aqueous phase was hydrolyzed with ß-glucuronidase and arylsulfatase and then extracted as described for enzyme-hydrolyzed urine samples (section (5)(a) of this DER). These extracts were concentrated to a small volume for separation by chromatographic methods.

Extracts were applied to silica gel TLC plates and developed in various solvent systems (Table 1). Spots and bands were scraped from the TLC plates and extracted with methanol. The extracts were concentrated and used for further separation by TLC and/or by reverse-phase HPLC. After the separation steps were completed, fractions were concentrated, applied to a prewashed silica gel TLC plate, and developed. Metabolites were eluted from the plates using an Eluchrom apparatus.

Characterization of metabolite structure was obtained by Fourier transform NMR (360 MHz); prior to analysis, samples were dissolved in deuterochloroform, and tetramethylsilane was added as an internal standard. Fast atom bombardment (FAB) mass spectrometry was used to verify molecular ion mass, and electron impact (EI) was used to confirm structures on the basis of fragmentation. For FAB, samples were dissolved in a thioglycerine or 3-nitrobenzylalcohol matrix and then bombarded with Xenon atoms. Spectrometry data obtained from the metabolites were compared with spectral data from synthesized reference compounds (Appendix A).

Qualitative and quantitative analysis of SAN 6[illegible]H metabolites present in urine, feces, and bile involved mixing extracted excreta samples with a solution of the reference standards and applying the mixture to TLC plates. A two-dimensional (2-D) TLC system was used to identify compounds corresponding to unchanged parent compound and to metabolites M1, M3 through M15, and M17 through M22; plates were developed twice with solvent system 6 (diethyl ether:dichloromethane:methanol:ammonium hydroxide (25% aqueous solution), 50:50:1:1 v/v v/v) and then twice with solvent system 11 (dichloromethane:methanol:formic acid, 88:2:0.5 v/v v). Metabolites M2 and M16 were identified via one-dimensional TLC using a single solvent system (solvent system 4, diethyl ether:ethyl acetate, 50:50 v/v). For metabolites M17, M18, and M19, TLC plates were developed in one direction with solvent system 11 (dichloromethane:methanol:acetic acid, 70:70:1.5 v/v v). Radioactive spots on TLC plates were detected

TABLE 1. TLC Solvent Systems Used for the Separation and Isolation of Urinary and Fecal Metabolites of Rats Dosed Orally with SAN 582H

solvent system no.	composition
SS 1	diethylether
SS 2	diethylether - ethyl acetate; 70 : 30 (v/v)
SS 3	diethylether - ethyl acetate; 50 : 50 (v/v)
SS 4	diethylether - methanol ; 95 : 5 (v/v)
SS 5	diethylether - dichloromethane - methanol; 60 : 35 : 5 (v/v)
SS 6	diethylether - dichloromethane - methanol - ammonium hydroxyde (25 % aqueous solution); 50 : 50 : 1 : 1 (v/v)
SS 7	ethyl acetate - n-hexane; 70 : 30 (v/v)
SS 8	ethyl acetate - methanol - formic acid; 95 : 5 : 0.5 (v/v)
SS 9	ethyl acetate - methanol - formic acid; 90 : 10 : 1 (v/v)
SS 10	dichloromethane - methanol; 95 : 5 (v/v)
SS 11	dichloromethane - methanol - formic acid; 98 : 2 : 0.5 (v/v)
SS 12	dichloromethane - methanol - acetic acid; 70 : 30 : 0.5 (v/v)

Source: CBI Table 10, CBI p. 41.

using photographic methods; spots of unlabeled reference material were visualized with a UV lamp (254 nm) and/or by spraying the plates with a mixture of 4-dimethylaminobenzaldehyde and sulfuric acid in an aqueous iron (III) chloride solution. Radiolabeled metabolites were quantified by scanning the TLC plates. For samples analyzed by 2-D TLC, the entire plate was scanned; for other samples, only one line (i.e., one sample) was scanned at a time. Data were analyzed using the software package NSCAN.

B. Protocols: A protocol was not included with this report.

12. REPORTED RESULTS:

A. [^{14}C]SAN 582H was readily absorbed and eliminated by all orally dosed rats (Tables 2 and 3). Elimination of radioactivity was essentially complete within 3 days after oral dosing for all groups, accounting for approximately 85 to 91% of the ^{14}C dose. Within 7 days, approximately 89 to 96.5% of the ^{14}C administered was recovered from the urine and feces (Table 2) or urine, feces, and bile (Table 3) of these animals. The iv-dosed rats excreted approximately 80 to 83 and 86 to 88% of the ^{14}C dose in the urine and feces within 3 and 7 days postinjection, respectively (Table 2). Urinary levels of ^{14}C were somewhat lower for high-dose rats than for other animals during the first 24 hours after compound administration. Urinary elimination of radioactivity was higher in female rats (47 to 63% of the ^{14}C dose) than in male rats (31 to 62%) (excluding the bile duct-cannulated animals); fecal levels of radioactivity, in turn, were lower in females (26 to 48%) when compared with males (30 to 62%) (Table 2). Urinary elimination of ^{14}C was highest in high-dose animals (62% males; 63% females); low- and repeated-dose animals excreted approximately 35% (males) and 48 to 53% (females) of the ^{14}C dose in the urine. Most of the radioactivity eliminated by iv-dosed females was recovered from the urine (49% of the ^{14}C dose versus 37% in the feces); in contrast, the urine and feces of iv-dosed males contained approximately 31 and 56% of the radioactivity administered, respectively. Biliary excretion of radioactivity was high, accounting for 82 and 75% of the ^{14}C dose given to males and females, respectively, at 72 hours after compound administration (Table 3).

B. [^{14}C]SAN 582H was extensively metabolized by rats. Twenty-two metabolites plus unchanged parent compound were isolated from the excreta of high-dose rats; all but one (fecal metabolite M22) were found in both the feces and the urine (Tables 4 to 7).

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Approximately 90 to 98% of the urinary radioactivity (29.6 to 59.2% of the ^{14}C dose) and 59 to 82% of the fecal radioactivity (19.2 to 47.1% of the dose) were recovered following the initial methanol extraction. The lowest excreta recoveries (59 and 65%) were for the feces of iv-dosed animals. Between 18 and 41% of the fecal ^{14}C (5.1 to 19.1% of the ^{14}C dose) was nonextractable following treatment with methanol; 1.7 to 12.5% of the fecal radioactivity (0.5 to 4.2% of the ^{14}C dose) remained bound following chemical hydrolysis and subsequent extractions with dichloromethane. Table 8 gives extraction recoveries for orally dosed rats.

Urine and feces contained both free and conjugated metabolites. For all experiments, the amount of free extractable metabolites (at pH 7 and pH 2) in the urine accounted for 14 to 20% of the ^{14}C dose given to males and 22 to 33% of that administered to females (Appendix Tables B1 to B4). The amount of conjugated metabolites (including both glucuronides and sulfates) recovered from the urine was dose and treatment dependent. Animals given either a single or repeated low dose (i.e., 10 mg/kg) of SAN 582H eliminated about 3% (males) and 10% (females) of the radioactive dose in the urine as conjugates within 72 hours after compound administration (Tables 4 and 7). High-dose males and females excreted approximately 23 to 24% of the ^{14}C dose in the urine as conjugates (Table 6). The primary metabolites recovered from the enzyme-treated urine of these animals were M5 (which represented about 6 to 7% of the ^{14}C dose), M1/M7 (about 5%), M2 (about 3%), M14 (approximately 2 to 4%), and M16 (about 1 to 2%). M17, a mercapturic acid metabolite, accounted for approximately 2 to 4% of the radioactive dose. Chemical hydrolysis of enzyme-treated urine samples released only 1 to 2% of the administered radiolabel, and of the ^{14}C in the remaining aqueous phase (7 to 9% of the dose), approximately 7 and 30% consisted of methylated and acetylated metabolites, respectively. Up to 16% of the ^{14}C dose, as extracted from the urine, was not identified, and an additional 11 to 15% consisted of polar fractions that were not further characterized. Trace amounts of the ^{14}C dose (<0.5%) were not extractable.

In contrast with urinary metabolite profiles, the amount of free extractable metabolites in feces (pH 7 and pH 2) was higher in males (16 to 30% of the ^{14}C dose) than in females (10 to 23.5%) and was lower for high-dose males and females (15.6 and 10.2%, respectively) when compared with other

TABLE 8. Distribution of Radioactivity in the Urine and Feces (0-72 Hr) of Rats Given Oral Doses of [^{14}C]SAN 582H[a]

	Percent of ^{14}C Dose Administered as:					
	10 mg/kg (single)[b]		1000 mg/kg (single)[b]		10 mg/kg (repeated)[c]	
Fraction	Males	Females	Males	Females	Males	Females
Extractable						
Identified						
Urine	9.4[d]	21.9	28.5	33.8	8.5	26.0
Feces	16.2	12.5	7.6	5.6	13.1	11.2
Total	25.6	34.4	36.1	39.4	21.6	37.2
Not identified						
Urine	9.4	9.2	16.1	11.7	11.0	12.6
Feces	12.7	13.4	8.7	6.3	18.6	9.8
Total	22.1	22.6	24.8	18.0	29.6	22.4
Polar Fractions						
Urine	13.6	13.0	15.0	14.7	12.4	11.2
Feces	15.1	7.6	6.9	7.3	14.6	6.6
Total	28.7	20.6	21.9	22.0	27.0	17.8
Total Extractable						
Urine	32.4	44.1	59.6	60.2	31.9	49.8
Feces	44.0	33.5	23.2	19.2	46.3	27.6
Total	76.4	77.6	82.8	79.4	78.2	77.4
Nonextractable						
Urine	0.4	0.5	0.1	0.1	0.4	0.4
Feces	10.2	11.3	5.7	5.1	12.1	9.5
Total	10.6	11.3	5.8	5.2	12.5	9.9
Total Excretion						
Urine	32.8	44.6	59.7	60.3	32.3	50.2
Feces	54.2	44.8	28.9	24.3	58.4	37.1
Total	87.0	89.4	88.6	84.6	90.7	87.3

[a]Prepared by the reviewers.

[b]Each animal received a single oral dose of 10 or 1000 mg [^{14}C]SAN 582H/kg.

[c]Each animal received 10 mg unlabeled SAN 582H/kg/day for 14 days followed by a single oral dose of 10 mg [^{14}C]SAN 582H/kg on day 15.

[d]Each value is the mean of six rats.

Source: CBI Tables 21, 23, and 24, CBI pp. 52, 54, and 55.

animals (20 to 30%, males; 13 to 23.5%, females) (Appendix Tables B5 to B8). Between 6 and 19% of the extractable fecal radioactivity was not identified; an additional 5 to 15% was "polar" material. The amount of nonextractable radioactivity in the feces accounted for 5 to 21% of the ^{14}C dose for all animals and was lowest (<6%) in high-dose rats. Chemical hydrolysis of the remaining aqueous phase and of the remaining fecal material yielded between 4 and 10% of the administered radioactivity. Excretion of conjugated metabolites in the feces was minimal for all groups, accounting for <2.5% of the ^{14}C dose administered. Major fecal metabolites included M13, which represented about 1.5 to 2% of the ^{14}C given to low-dose rats; M16, which accounted for 2 to 4% of that administered to all animals; and M1/M7, which accounted for approximately 2 to 5% of the radioactivity given to low- and repeated-dose rats. Most other fecal metabolites represented ≤1% of the administered radiolabel.

Between 11 and 16% of the ^{14}C dose was initially extracted from the bile (CBI p. 32); an additional 32 to 36% was conjugated, and approximately half of this consisted of glucuronide and/or sulfate conjugates of SAN 582H (Appendix Table B9). A large amount of the radioactivity in the bile (i.e., 48 to 51% of the ^{14}C dose) was "polar" material, and 6 to 11% was extractable but not identified. Only a small fraction of the administered radioactivity (<1.5%) was not extractable.

Between 1 and 2% of the test material was excreted unchanged, primarily in the feces (Tables 4 to 7).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Single low, single high, and repeated low oral doses of SAN 582H were readily absorbed and eliminated by male and female rats. Elimination of radioactivity was essentially complete within 3 days after dosing with ^{14}C-labeled test material, and more than 90% of the ^{14}C dose was excreted in the urine and feces or urine, feces, and bile within 7 days. The total amount of the radiolabel eliminated was independent of dose and sex. However, orally dosed females excreted more of the radioactive dose in the urine (47 to 63%) and less in the feces (26 to 48%) than males (31 to 62% and 30 to 62%, respectively. In addition, urinary levels of ^{14}C were much higher for high-dose rats (62 to 63% of the total dose) than for all other groups (31 to 53%), indicating possible saturation of biliary excretion in high-dose animals (see additional discussion below). Recovery of 75 to 82% of a single oral dose of 10 mg

[^{14}C]SAN 582H/kg in the bile demonstrated that the test material and its metabolites were excreted primarily via the liver.

SAN 582H was readily and extensively metabolized by all animals. In addition to small amounts (≤2% of the ^{14}C dose) of unchanged parent compound, 22 metabolites combined accounted for approximately 40% of the radioactivity administered. An additional 20% consisted of several extractable but unidentified metabolites, and the remaining 20% was described as a mixture of very polar plus conjugated metabolites that were not further characterized.

The major metabolic pathways for SAN 582H involve glutathione conjugation followed by (1) gamma-glutamyltransferase transpeptidation and N-acetylation of the resulting cysteinyl derivative to form mercapturic acid (metabolite M17) (Figure 1) or (2) hydrolysis to the mercaptan, which is further metabolized to the intermediate PL 36-88 by S-methylation; to metabolite M21 by cyclization and hydroxylation; or to M22 by dimerization (Figure 2). The S-methylated intermediate PL 36-88 appears to undergo O-demethylation to form the hydroxylated metabolite M1; PL 36-88 may also be oxidized to the sulfoxide M13 (Figure 3). Further metabolism of M13 involves oxidation of the 2-methyl group on the thiophene ring to form the hydroxymethyl metabolite M16; O-demethylation to M2 followed by oxidation to the hydroxy-sulfone metabolite M14 and subsequent oxidation to the carboxylic acid M19; or oxidation to the sulfone M10, which may, in turn, undergo O-demethylation to M14 and subsequent oxidation to M19 (Figure 3). The study author suggested that the S-methyl group on metabolite M1 is oxidized to the sulfoxide M2, which is then oxidized to metabolites M14 and M19 as described above; the methylethyl moiety on M1 is oxidized to carboxylic acid (Figure 3).

The minor metabolic pathways of SAN 582H involve direct breakdown of the parent compound. The predominant reactions include (1) demethylation to metabolite M7 (Figure 4) and (2) oxidation of the 2- or 4-methyl group on the thiophene ring to produce metabolite M5 and the proposed intermediate PL 77-88, respectively (Figure 5). Cyclization of metabolite M7 results in the formation of M9, which, in turn, undergoes hydroxylation to produce M15 (Figure 4); dehydration of the newly hydroxylated ring of M15 gives M8 (Figure 4). Cyclization of PL 77-88 and M5 results in metabolites M20 and M6; respectively (Figure 5).

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Additional minor pathways for the metabolism of SAN 582H involve hydrolysis, i.e., replacement of the chlorine by a hydroxyl group to produce M11; direct oxidation of the sulfur in the thiophene ring to produce the unstable sulfoxide, metabolite M4; and reductive dechlorination to M3 and subsequent demethylation to M12 (Figure 6).

The study author concluded that, in general, the metabolic pathways for SAN 582H were independent of sex and dose. However, glutathione conjugation appeared to be significantly higher in low-dose animals than in high-dose rats: the ratio of the amounts of the identified metabolites formed via glutathione conjugation versus by direct transformation of the parent compound was 2:1 for the high-dose group and 4:1 for low-dose animals. The lower ratio for the high-dose group was attributed to possible saturation of glutathione conjugation at the 1,000-mg/kg dose level.

B. A quality assurance statement, signed and dated November 16, 1989, and a statement of compliance with Good Laboratory Practices (GLPs), signed and dated November 13, 1989, were included in the report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

This study provides supplementary information on the metabolism of SAN 582H in male and female rats for all treatment regimens required by EPA (Guideline 85-1): single low, single high, and repeated low oral dosing. Although the study author reported that all major metabolites were identified, between 61 and 81% of the ^{14}C dose was not identified or characterized, and data supporting the statement that this fraction of the dose consisted of primarily minor polar and/or conjugated metabolites were not presented. The absence of these data and the inability to characterize up to 30% of the radioactive dose that was extractable may indicate inadequate methodology. The report would be strengthened and the conclusions better supported through the inclusion of complete chromatographic results.

Orally administered [^{14}C]SAN 582H is readily absorbed and extensively metabolized by rats. Elimination of radioactivity was essentially complete within 3 days after dosing and the primary route of elimination, based on bile duct cannulation studies, was via the liver. As noted by the

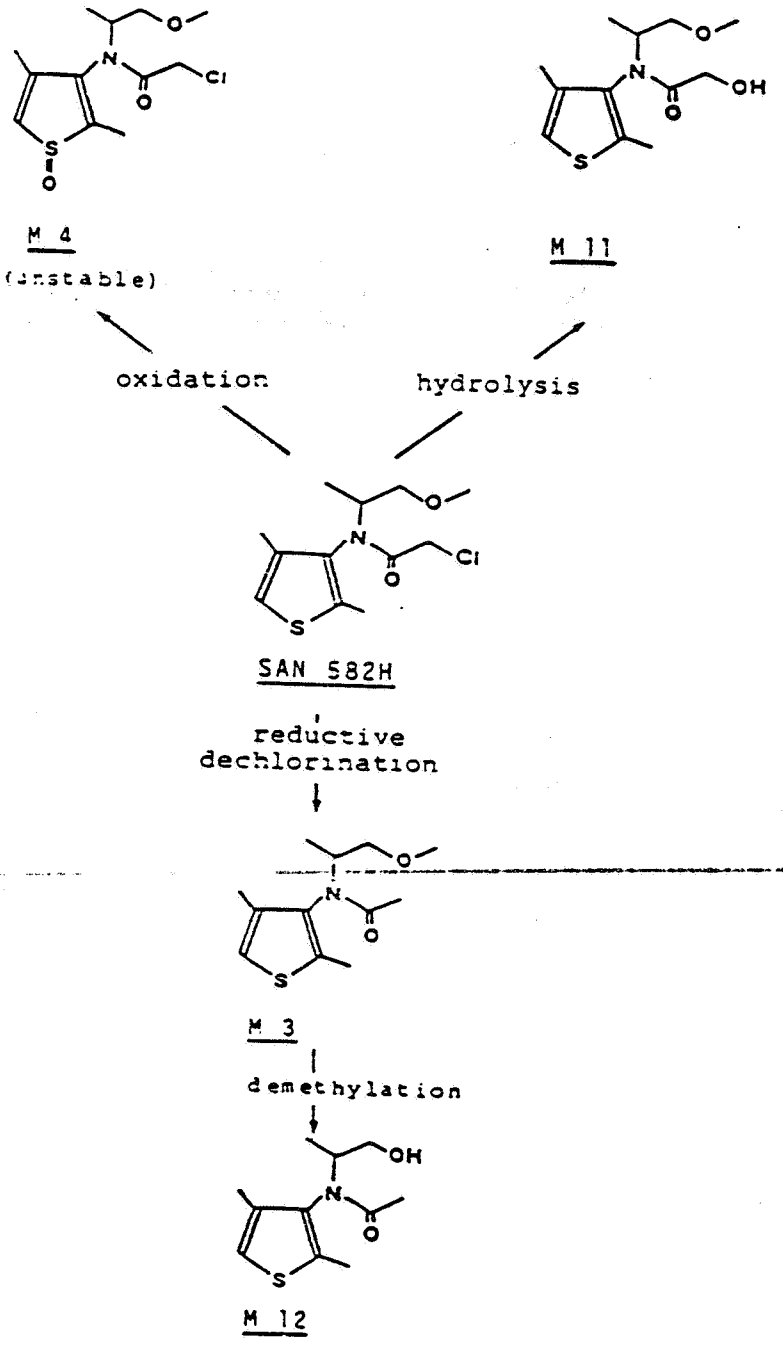

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FIGURE 6. Proposed minor metabolic pathways for SAN 852H in rats.

Source: CBI Figure 1f, CBI p. 62.

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study author, the total amount of radiolabel eliminated was independent of dose level, dosing regimen, and sex. However, orally dosed females tended to eliminate more of the ^{14}C administered in tne urine (47 to 63%) and less in the feces (26 to 48%) than males (31 to 62% and 30 to 63%, respectively). In addition, although urinary elimination of radioactivity by high-dose rats was somewhat slow during the first 24 hours postdosing, these animals eliminated more of the total dose (i.e., 62 to 63%) in the urine than all other groups (31 to 53%) within 72 hours after compound administration. These data suggest that biliary excretion of SAN 582H and its metabolites may have been saturated in females and following administration of the high dose (1000 mg/kg) to both males and females.

On the basis of the metabolites identified, the data presented support the conclusions of the study author that SAN 582H is extensively and essentially completely metabolized, and that the primary metabolic pathway for SAN 582H involves glutathione conjugation followed by breakdown of the glutathione conjugate via the mercapturic acid pathway or hydrolysis of the thio bond of the mercapta: (which is then methylated and oxidized to the methylsulfoxide and methylsulfone). Overall, this study was classified as supplementary.

Items 15 and 16--see footnote 1.

APPENDIX A

Reference Standards
for SAN 582H Metabolites
(CBI Table 9, CBI pp. 33-40)

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APPENDIX B

Distribution of Free and Conjugated
Metabolites in the
Urine, Feces, and Bile

(CBI Tables 12-20, CBI pp. 43-51)

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