6-16-95

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

JUN 16 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Kathon® 287T Industrial Microbicide (707-EEU)
Study Reviews and Data Requirements

TO: Valdis Goncarvos
PM Team Reviewer (31)
Registration Division (7505C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 6/12/95
Toxicology Branch II, Section II,
Health Effects Division (7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 6/13/95
Section II Head, Toxicology Branch II
Health Effects Division (7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 6/15/95
Chief, Toxicology Branch II/HFAS/HED (7509C)

Registrant: Rohm & Haas Company
Chemical: 4,5-dichloro-2-n-octyl-3-(2H)-isothiazolone
Synonym: Kathon® 287T Industrial Microbicide
Caswell No.: 314B
Submission No.: S478172
DP Barcode: D212609
PC Code: 128101
Identifying No.: 000707-EEU KATHON 287T
Case No.: 192535
MRID No.: 434716-01 through 434716-10
Action Requested: Review the data as requested in previous reviews; also determine if any additional data are required and whether all data requirements have been satisfied. The cover letter is enclosed for your comment;N new uses added to this chemical. Are they supported by the data you have? If not, itemize what else you need.

Comment: The Registrant has submitted several studies in support of the registration of Kathon® 287T Industrial Microbicide [listed below], and these have been reviewed [DER's appended].

(1) **MRID # 434716-01** RH-287 Technical Acute Oral Toxicity Study in Male and Female Mice. The acute oral lethal dose [LD_{50}] of RH-287 Technical in mice was 567 mg/kg for the combined sexes, 701 mg/kg for males, and 451 mg/kg for females. TOXICITY CATEGORY: II

[default, based on results in females]. This study is classified Core Minimum, and it satisfies the guideline requirements [§81-1] for an acute oral toxicity study in mice.

(2) **MRID # 434716-02** RH-287 Technical Acute Inhalation Toxicity Study in Rats. Due to unexplained discrepancies between the nominal and analytical concentrations of the doses tested, no LC_{50} can be calculated. No repeat acute inhalation study on RH-287 Technical is recommended due to its irritant properties. This study does not satisfy the guideline requirement (81-3) for an acute inhalation study in rodents, but it is classified Acceptable. A Toxicity Category I will be used.

(3) **MRID # 434716-03** RH-287 Technical: Three-Month Dietary Toxicity Study in Rats. Under the conditions of the study, oral administration of RH-287 Technical (98.8%) via the diet to Crl:CD®BR rats [10/sex/group] for 13 weeks at dose levels of 0, 100 ppm [6.2 ♂/7.2 ♀ mg/kg], 500 ppm [32.5 ♂/36.7 ♀ mg/kg], 1000 ppm [60.7 ♂/74.7 ♀ mg/kg], and 4000 ppm [248.2 ♂/278.4 ♀ mg/kg] resulted in decreased body weight throughout the study at the high-dose level in both sexes {♂♂ 86%/♀♀ 80% of control at 13 weeks}, decreased overall body-weight gain {♂♂ 79%/♀♀ 77% (4000 ppm)/♀♀ 85% (1000 ppm) of control}, decreased food consumption at 4000 ppm for both sexes and at 1000 ppm in females initially, decreased water consumption at 4000 ppm, changes in several hematology/blood chemistry parameters consistent with chronic, low-level blood loss, and microscopic, treatment-related, changes in the forestomach {↑ mucosal erosion/ulceration, submucosal edema, acute inflammation, hyperplasia of the mucosal epithelium, and/or hyperkeratosis}. **The NOEL can be set at 500 ppm [32.5 ♂♂ /36.7 ♀♀ mg/kg/day], the LEL at 1000 ppm [60.7 ♂♂ /74.7 ♀♀ mg/kg/day], based on microscopic lesions in the forestomach in males and decreased body weight/gain and food consumption, decreased serum triglyceride levels, and microscopic lesions in the forestomach in females.** This study is classified Core Minimum, and it satisfies the guideline requirement [82-1(a)] for a subchronic oral toxicity study in rodents.

(4) **MRID # 434716-04** RH-287 Technical Oral (Gavage) Developmental Toxicity Study in Rats. Under the conditions of the study, dose levels of 0, 10, 30, and 100 mg Kathon 287 Technical (98.8%)/kg of body weight/day administered to 25 pregnant Crl:CD®BR rats/group during Days 6-15 of gestation resulted in (1) the death of 1/25 dams at the 100 mg/kg/day level, (2) decreased body-weight gain [73% of control] at the high-dose level during the dosing period with a concomitant decrease [88-90% of control] in food consumption, (3) decreased food consumption at the mid-dose level during the last week of dosing; (4) transient scant feces, soft feces, and/or diarrhea at the mid- and high-dose levels, and (5) an increased number of litters with fetuses with wavy ribs at the high-dose level. Pregnancy rate was comparable among the groups, and there were no statistically significant differences in the number of corpora lutea/dam, implantations/dam, live fetuses/dam, resorptions/dam, dead fetuses/dam, or in pre- or post-implantation losses, litter weight, net body-weight change in the dams, or fetal body weight (combined and per sex). There were no fetal external

variations or developmental retardations, and there were no fetal soft tissue developmental retardations. Neither control group displayed fetal external malformations, fetal soft tissue malformations, and fetal soft tissue variations. There was an increase in the number of fetuses [21 vs 2] at the high-dose level with wavy ribs, along with an increase in the severity of the finding and a statistically significant increase in the number of high-dose litters [11 vs 1] with fetuses with wavy ribs compared to the concurrent control. No other statistically significant differences were attributed to treatment. **The NOEL for maternal effects can be set at 10 mg/kg/day, the LEL at 30 mg/kg/day, based on decreased food consumption during the dosing period and transient scant feces, soft feces, and/or diarrhea. The NOEL for developmental toxicity can be set at 30 mg/kg/day, the LEL at 100 mg/kg/day, based on the increased number of litters with fetuses with wavy ribs.** This study is classified Core Minimum, and it satisfies the guideline requirement [83-3(a)] for a developmental toxicity study in the rat.

(5) **MRID # 434716-05** RH-287 Technical: Salmonella typhimurium Gene Mutation Assay (Ames Test). There was no evidence of a mutagenic response at any dose either with or without S9 activation. This study is classified Acceptable, and it satisfies the guideline requirement [84-2] for a microbial gene mutation assay.

(6) **MRID # 434716-06** Test for Chemical Induction of Gene Mutation at the HGPRT Locus in Cultured Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation. There was no evidence of a mutagenic response at any dose either with or without S9 activation. This study is classified Acceptable, and it satisfies the guideline requirement [84-2] for an in vitro mammalian cell gene mutation assay.

(7) **MRID # 434716-07** Test of Chemical Induction of Chromosome Aberration in Cultured Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation. The evidence is not sufficient to conclude that RH-287, tested up to cytotoxic levels, induced a clastogenic response in the presence or absence of S9 activation. This study is classified Acceptable, and it satisfies the guideline requirement [84-2] for an in vitro mammalian chromosome aberration assay.

(8) **MRID # 434716-08** RH-287 Technical: Micronucleus Assay in CD-1 Mouse Bone Marrow Cells. A slight but dose-related increase in MPES was observed in the males of the mid- and high-dose groups at the 24-hour sacrifice. The increase was significant at 325 mg/kg. However, the findings are only suspect and do not provide sufficient evidence to classify RH-287 as clastogenic/aneugenic in this test system. This issue can only be resolved by exposing the test animals to the MTD. This study is classified as Unacceptable, and it does not satisfy the guideline requirement [84-2] for a micronucleus assay.

(9) **MRID # 434716-9** ^{14}C-RH-287: Pharmacokinetic Study in Rats. Rats [Crl:CD®BR; 4 or 5/sex/group] were administered ^{14}C-RH-287 [4,5-

labeled] orally single low [20 mg/kg], single high [250 mg/kg], or 14 consecutive unlabeled low [20 mg/kg/day] followed by a single ^{14}C low [20 mg/kg] doses of RH-287 Technical. Following single exposures, essentially all of the ^{14}C was excreted in the feces and urine, with >81% {**feces:** ♂♂ ≈74% [low]/≈81% [high]; ♀♀ ≈77% [low]/≈71% [high]; **urine:** ♂♂ ≈13% [low]/≈10% [high]; ♀♀ ≈16% [low]/≈10% [high]} being eliminated within 2 days. No sex difference was observed. Similarly following a 14-day repeated 20 mg/kg/day dose, >60% [**feces:** ♂♂ ≈53%/♀♀ ≈43%; **urine:** ♂♂ ≈ 17%/♀♀ ≈ 17%] of a single 20 mg/kg ^{14}C dose was eliminated within 2 days. Radiolabel was distributed to all tissues measured. The concentration of radiolabel remaining in the tissues at 96 hours was dose-related and represented ≈ 0.3% ♂♂/<0.5% ♀♀ of the administered dose. At 4 days post dose, the highest percentage of the dose was displayed in the liver, kidney, and stomach. The overall recovery of radiolabel following single-dose exposure was 90%-96%. Overall recovery following repeated dosing was lower [64%-74%], but this was attributed to differences in fecal homogenate preparation. Pretreatment for 14 days did not alter the elimination of the radiolabel. It does not appear that RH-287 Technical would bioaccumulate significantly. This study is classified Acceptable. The identification and characterization of the metabolites is to be reported in a separate report. This study does not satisfy the guideline requirement (85-1) for a metabolism study, but with the submission of an acceptable metabolite identification report, this data requirement will be satisfied.

(10) **MRID # 434716-10** ^{14}C-RH-287 Dermal Absorption Study in Male Rats. The test material was administered in acetone, an inappropriate vehicle for dermal absorption studies, since it has been found to produce significant qualitative and quantitative effects on the absorption process. This study is classified Unacceptable.

DISCUSSION: You indicated that the Registrant has added a "whole range of new uses to this technical", and you asked whether they are supported by the data available. As indicated in previous reviews on this microbicide, with the exception of a 90-day inhalation study, the data available satisfy the data requirements for an antimicrobial in the **low** exposure category. TB II is not aware of any OREB assessment [TB II memo dated 3/30/94 indicated that this is needed] of the previous uses, and the current data package does not appear to have been submitted to OREB for their review. You indicated via cc:mail in response to my inquiry that an OREB review was requested [D196245 on 10/20/93] previously, but it has not been completed. You also indicated that my 3/30/94 memo may not have been provided to OREB. Because of a lack of exposure information, TB II cannot comment on whether the new uses are supported by the existing toxicology data base or whether additional data are required. With regard to the 90-day inhalation study requirement, based on the requirements of other microbicides with several of the uses listed for Kathon 287 Technical, a 90-day inhalation study is required.

011587

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 4/17/95
Section II, Tox. Branch II (7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 4/17/95
Section II Head, Tox. Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral - Mice (§81-1) TOX. CHEM. NO.: 314B

MRID NO.: 434716-01 Shaughnessey #: 128101

TEST MATERIAL: RH-287T Technical

SYNONYMS: 4,5-dichloro-2-n-octyl-3(2H) isothiazolone

STUDY NUMBER: Report # 94R-003; Protocol # 94P-003

SPONSOR: Rohm and Haas Company

TESTING FACILITY: Rohm and Haas Company Toxicology Department

TITLE OF REPORT: RH-287 Technical Acute Oral Toxicity Study in Male and Female Mice

AUTHORS: MF Lutz and JR Parno

REPORT ISSUED: August 30, 1994

QUALITY ASSURANCE: A quality assurance statement and a Good Laboratory Practice compliance statement were provided.

EXECUTIVE SUMMARY: Under the conditions of the study [6 Crl:CD-1® (ICR)BR mice/sex/group; dose groups of 100, 250, 500, 1000, and 2000 mg/kg], **the acute oral lethal dose [LD_{50}] of RH-287 Technical in mice was 567 mg/kg for the combined sexes, 701 mg/kg for males, and 451 mg/kg for females.** This study is classified Core Minimum, and it satisfies the guideline requirements [§81-1] for an acute oral toxicity study in mice.

TOXICITY CATEGORY: II [default, based on results in females]

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A. MATERIALS

1. Test compound: RH-287 Technical; Description: tan to brown solid; Batch #: Lot #: 4086; Purity: 99.8% 4,5-dichloro-2-n-octyl-3(2H) isothiazolone; Source: Sponsor..

2. Test animals: Species: mouse; Strain: Crl:CD-1®(ICR)BR; Age: ≈ 32 days old at dosing; Weight: fasting weights ♂♂: 25-38 g, ♀♀: 24-30 g; Source: Charles River Portage, Portage, MI.

B. STUDY DESIGN

1. Methodology: Six male and six female mice per dose level were administered the test material [corn oil] via gavage [dose level: 100, 250, 500, 1000, and 2000 mg/kg] in a dosage volume of 10 mL/kg, following a 3-hour fast. The mice were selected from a healthy stock population and assigned to the groups using a computer-generated sequence of random numbers. Mice were housed 2 per cage during quarantine [length not stated] and one/cage during study. Water was available ad libitum, and PMI Certified Rodent Diet 5002(C) was provided. All mice were observed for signs of toxicity at 1, 2, and 4 hours post dose and once a day thereafter for 14 days. Body weight was recorded on day 0 prior to dosing and on Days 7 and 14. All surviving mice were sacrificed on day 14, and necropsies were performed on all decedents [as they occurred] and surviving mice [at termination]. The necropsy consisted of a gross examination of all organs in situ.

2. Dose preparation: The test material and corn oil were heated in an oven [60°C], and an appropriate amount of liquified test material was mixed with heated corn oil to obtain a solution. The solutions were kept heated and stirred during dosing, and the dosing suspension was gavaged at 37°C. The doses were administered within 90 minutes of preparation. No data were provided with respect to stability, homogeneity, or concentration of the test material suspensions.

3. Statistical procedures: The mortality incidences of both sexes were compared across doses with a categorical data modeling procedure using SAS CATMOD [SAS Institute Inc., SAS User's Guide: Statistics, Version 6 Edition, pp 405-517 (1990)]. The LD_{50}, 95% confidence limits, and slope were calculated from the logarithm of the doses and the incidences of death using a SAS PROBIT procedure [ibid. pp 1325-1350], based on the method of DJ Finney [Probit Analysis, 3rd Edition, London: Cambridge University Press, 1971].

C. RESULTS

Deaths occurred in both sexes at dose levels of 500 mg/kg and

above [Table 1]. All deaths [with one exception] were considered related to treatment and occurred within 3 days of dosing. Clinical signs of diarrhea and brown-stained anal-genital area were observed at ≥ 500 mg/kg in both sexes and attributed to the use of corn oil as vehicle. Soft and/or scant feces, passiveness, tremors, and ataxia were the clinical signs attributed to the test material. These were observed at dose levels of 1000 and 2000 mg/kg in both sexes and at 500 mg/kg in females. Decreased body-weight gain was observed in both sexes at 500 mg/kg, but both sexes at the two lowest dose levels showed comparable body-weight gains [Table 2]. Decedents displayed black material in the stomach, reddened glandular portion of the stomach and intestines, and mottled liver at necropsy. Because no statistically significant difference was observed between the sexes, the author calculated the LD_{50} from the combined mortality incidence data. However, at the 500 mg/kg dose level, 4 of the 6 females died, and the Registrant was requested to submit an estimate for the sexes separately [Table 2]. The Registrant emphasized the fact that this study was performed to determine dose levels to be used in a mouse micronucleus assay.

Dose [mg/kg] sex	Table 1. Mortality [# of deaths/# dosed]				
	100	250	500	1000	2000
males	0/6	0/6	1/6	5/6	6/6
females	0/6	0/6	4/6	6/6♦	6/6

♦ one euthanized for humane reasons [had been misdosed]

Table 2. Estimated LD_{50} and 95% Confidence Limits		
Group	Estimated LD_{50}	Lower/Upper 95% Limit
Combined sexes	567 mg/kg	433/733 mg/kg
Males	701 mg/kg	445/1248 mg/kg
Females	451 mg/kg	172/807 mg/kg

Day Dose [mg/kg] Sex	Table 3. Body-Weight Change [grams]				
	100	250	500	1000	2000
Males					
0-7	2.7	2.0	-0.4	-5.0♦	-
7-14	1.3	1.8	2.0	7.0	-
0-14	4.0	3.8	1.4	2.0	-
Females					
0-7	2.3	2.5	0.5♥	-	-
7-14	0.7	0.5	1.0	-	-
0-14	3.0	3.0	1.5	-	-

♦ one mouse; ♥ 2 mice

D. CONCLUSIONS

Under the conditions of the study, **the acute oral lethal dose [LD_{50}] of RH-287 Technical in mice was 567 mg/kg for the combined sexes, 701 mg/kg for males, and 451 mg/kg for females.** This study is classified Core minimum, and it satisfies the guideline requirements (§81-1) for an acute oral toxicity study in mice.

011587

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 4/11/95
Section II, Tox. Branch II (7509C)
Secondary Reviewer: K. Clark Swentzel K Clark Swentzel 4/11/95
Section II Head, Tox. Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute inhalation - rat TOX. CHEM. NO.: 314B

MRID NO.: 434716-02 Shaughnessey #: 128101

TEST MATERIAL: RH-287T Technical

SYNONYMS: 4,5-dichloro-2-n-octyl-3(2H) isothiazolone

STUDY NUMBER: Report # 93R-217; Protocol # 93P-217

SPONSOR: Rohm and Haas Company

TESTING FACILITY: Rohm and Haas Company Toxicology Department

TITLE OF REPORT: RH-287 Technical Acute Inhalation Toxicity Study in Rats

AUTHORS: HJ Bernacki, Jr. and JS Ferguson

REPORT ISSUED: October 24, 1994

QUALITY ASSURANCE: A quality assurance statement and a Good Laboratory Practices Compliance statement were provided.

CONCLUSIONS: Under the conditions of the study, exposure of rats to aerosol/vapor of RH-287 Technical at [analytical] dose levels of 0.12, 0.20, 0.23, and 0.46 mg/L [nominal concentrations: 0.12, 0.05, 0.12, and 0.42 mg/L, respectively] for 4 hours [nose only] resulted in the death of all rats of both sexes at the highest dose level and of 5 of 6 males but none of the females at the 0.20 mg/L dose level. Based on the nominal concentrations, these dose levels represent the highest and lowest doses tested. Due to unexplained discrepancies between the nominal and analytical concentrations, no LC_{50} can be calculated. No repeat study on RH-287T Technical is recommended due to its irritant properties.

Classification: Acceptable. This study does not satisfy the guideline requirement (81-3) for an acute inhalation study in rodents.

TOXICITY CATEGORY: I [default, based on results in males]

A. MATERIALS

1. Test Compound: RH-287 Technical; Description: tan/brown solid; Batch #: Lot # 4086; Purity: 98.8% 4,5-dichloro-2-n-octyl-3(2H) isothiazolone; Source: Sponsor.

2. Test Animals: Species: rat; Strain: Crl:CD®BR; Age: ♂♂ ≈7 weeks/♀♀ ≈9 weeks; Weight: ♂ 204-300 g; ♀ 191-269 g at dosing; Source: Charles River-Kingston, Stone Ridge, NY.

3. Statistics: Because of an apparent inversion in the dose response relationship observed, the standard probit method [SAS CATMOD, 1985] could not be used to calculate the LC_{50} for the test material. Thompson's Moving Average method was used to estimate the LC_{50}.

B. STUDY DESIGN

1. Methodology: Rats were assigned (randomly selected from stock population; one week quarantine) to one of four exposure groups [6/sex]. The protocol indicates that, based on the results of previous inhalation studies on formulations of the test material, the initial exposure target concentration would be 0.1 mg/L. The nominal concentrations were 0.12, 0.11, 0.42, and 0.05 mg/l [listed in the order tested]. The analytical exposure concentrations were 0.23, 0.12, 0.46, and 0.20 mg/L, respectively. The length of exposure was 4 hours. The rats were housed individually and were provided Purina Certified Rodent Chow® 5002 and water ad libitum during nonexposure period. During exposure (nose-only inhalation), the rats were placed in individual restraining tubes [6" x 2" PVC pipe with neoprene stopper at rear and a plastic funnel in front, Figure 1, appended] inside an exposure chamber [240-L Plexiglas®and stainless steel; Figure 2, copy appended].

2. Generation and Monitoring of Test Atmosphere and Exposure: Test material [undiluted] was melted at ≈50°C and placed in a 3-necked flask heated to maintain a temperature of ≈60°C. Test atmospheres for the four exposure groups were generated using a Laskin generator [0.135 inch opening], which was inserted into the 3-necked flask and operated with compressed air heated to ≈90°C [except during the 0.20 mg/L exposure when the air was heated to 50°C]. The various chamber concentrations were achieved by varying the chamber airflow rate. A two impinger train located outside the chamber was used for sampling the chamber atmosphere in order to determine the analytical concentrations of test material achieved [Figure 3, appended]. The chamber atmosphere was drawn through the impingers [flow rate of 0.7 L/min for 17 minutes] Three samples for each exposure group were collected. Each collecting solution was analyzed by High Performance Liquid Chromatography [HPLC], and the results were reported in ppm RH-287 Technical. The following equations were used to convert to airborne concentrations in mg/L of chamber atmosphere:

$$\text{mg RH-287 ai} = \text{ppm ai} \times \frac{\text{g of solution}}{1000}$$

$$\text{mg/L RH-287 in air} = \frac{\text{mg of RH-287 (ai)}}{\text{(sample duration} \times \text{flow rate}}$$

Additionally, gravimetric determinations of the aerosol concentration were made periodically [5-8 samples/exposure] during exposure. A known volume of chamber atmosphere was drawn through a pre-weighed glass fiber filter, and the change in weight of the filter divided by the sample volume yielded the aerosol concentration. NOTE: HPLC analytical results indicated that the actual test material concentration was 2-3 fold higher than the aerosol concentration measured gravimetrically, which indicates that a significant portion of the exposure atmosphere contained vaporized test material that could not be quantified using a gravimetric method. Therefore, gravimetric results were used only to monitor the exposure concentrations. The reported HPLC analytical results reflect total exposure concentrations of a mixture of RH-287 Technical aerosol and vapor.

During each exposure, **particle size distribution** was determined twice by drawing samples of chamber atmosphere through an 8-stage Andersen cascade impactor fitted with a glass fiber collection disc. The calculation of **mass median aerodynamic diameter** [MMAD] and **geometric standard deviation** [GSD] was performed by computer using a log-probit regression analysis program [Hagan, 1980]. The respirable fraction was calculated from the MMAD and GSD in the RFB program [Moss and Baldwin, 1983] on a programmable calculator, which defines "respirable fraction" as that fraction of an aerosol that would pass a size-selector [American Conference of Governmental Industrial Hygienists (ACGIH)] with the following characteristics: 90% of $\leq$ 2.0-μm particles; 75% of 2.5-μm particles; 50% of 3.5-μm particles; 25% of 5.0-μm particles; and 0% of $\geq$ 10-μm particles will pass through the selector [Lippman, 1978]. Chamber airflow [monitored continuously during exposure], temperature, and % relative humidity were monitored during each exposure. With respect to oxygen levels, it was stated that the lowest chamber flow rate provided $\approx$10 air changes/hour, which would be expected to maintain at least 19% oxygen in the chamber.

RESULTS

There were four separate exposure days, each testing a different concentration of test material. The mean temperature in the exposure chambers ranged from 21.7-22.2°C, and the relative humidity range reported was 32.8-54.7%. Chamber flow rate and aerosol equilibrium time [t_{99}] are listed below. The longest calculated 99% aerosol equilibrium time [28 minutes] exceeded 10% of the total exposure time as per protocol, but the increase [11.7% of total exposure time] was not considered to have affected the results. After the chamber was turned off, the rats remained in the nose-only tubes in the exposure chamber for a time at least as long as the calculated t_{99}. A summary of the analytical determinations and size distribution are listed below in Table 1. The order of the concentrations is the order in which they were tested. It is to be noted that the nominal concentration in each case is **lower than** the analytical concentration, which is "unusual" at

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best. No discussion/explanation was provided for this finding.

Table 1. Summary of Exposure Parameters

Analytical Concentration [mg/L]	Nominal Concentration [mg/L]	Chamber Flow Rate [L/min]	t_{99} [minutes]	Mass Median Aerodynamic Diameter (μm)	Geometric Standard Deviation (μm)	Mean respirable fraction
0.23	0.12	110	10	1.4±0.07	1.7±0.35	88.0±0%
0.12	0.11	60	18	2.1±0.64	1.9±0.14	71.5±9.19%
0.46	0.42	40	28	1.5±0	1.7±0.35	83.5±4.95%
0.20	0.05	100	11	1.3±0	1.8±0.64	84.5±6.36%

3. Clinical Observations: All rats were weighed just prior to exposure [day 0] and on days 7 and 14 of the 14-day post-dose observation period. Each rat was observed for signs of toxicity during exposure and upon removal from the exposure chamber. Thereafter, the rats were observed twice daily for mortality, morbidity, and clinical signs during the observation period [except holidays, weekends, and on day 14 when they were observed once a day].

RESULTS

Survival and Clinical Observations

Deaths occurred at all dose levels [Table 2], and all were attributed to the test material. None of the females exposed to the 0.20 mg/L [analytical] dose died, but 5 of the 6 males died at this dose level. All groups displayed signs of respiratory irritation [gasping and slight to severe rales]. Unkempt appearance, red-stained eyes and muzzle, scant feces and yellow-stained anogenital area were also observed is some of the groups. All surviving rats [one exception: ♀ at 0.20 mg/L exhibited slight rales through day 13 post exposure] appeared normal by day 7. NOTE: The order in which the data are presented corresponds to the order in which the doses were tested.

Dose [mg/L] sex	Table 2. Mortality [# of deaths/# dosed]			
	0.23	0.12	0.46	0.20
males	1/6	2/6	6/6	5/6
females	2/6	3/6	6/6	0/6

4. Body Weight

All animals, except one 0.20 mg/L female, who lost 1 gram during the 0-7 day interval, gained weight during the study. Body-weight gains in the male groups were comparable [except the 0.46 mg/L males who all died by day 1]. In females, those at the 0.20 mg/L dose level displayed a decrease in body-weight gain compared to the other groups [except the 0.46 mg/L females who all died by day 2].

Day Dose [mg/L] Sex	Table 3. Body-Weight Change [grams]			
	0.23	0.12	0.46	0.20
Males				
0-7	36	39	-	33♦
7-14	58	66	-	60
0-14	94	104	-	93
Females				
0-7	19	20	-	7
7-14	17	20	-	9
0-14	37	40	-	16

♦ one male

5. Sacrifice and Pathology

After the 14-day observation period, all survivors were sacrificed [anesthetized, exsanguinated from abdominal aorta] and necropsied. Visual examinations of the external structures and body orifices were performed, and thoracic and abdominal organs were examined in situ.

RESULTS

Gross Pathology: There were gross pathological findings in the lungs that were attributed to treatment. These included slight to severe redness on all lobes and few to multiple red pinpoint foci on all lobes. Gas-filled stomachs were observed in several rats of both sexes, which is consistent with respiratory distress [gasping and rales] and probably due to swallowing air while breathing.

Table 4. Gross Pathology Findings				
Lesion/Sex/Dose [mg/L]	0.23	0.12	0.46	0.20
MALES				
Lungs				
redness on all lobes				
slight	-	1	-	-
moderate	-	-	1	-
severe	1	2	5	5
red pinpoint foci-all lobes				
few	1	-	-	-
multiple	-	-	-	1
few pin point foci-left lateral lobe	-	1	-	-
Stomach gas-filled	0	1	3	3
no gross observations	4	3	0	1
FEMALES				
Lungs				
redness on all lobes				
slight	1	2	-	5
moderate	-	-	2	1
severe	2	3	4	-
red pinpoint foci-all lobes				
few	-	-	-	-
multiple	-	-	-	1
Stomach gas-filled	0	1	2	0
no gross observations	3	1	0	0

Histopathology: No histopathology was performed in this study.

C. DISCUSSION

Exposure of rats to analytical concentrations of 0.46, 0.20, 0.12, and 0.23 mg/L of RH-287 Technical aerosol/vapor for 4 hours [nose-only] resulted in deaths of 100%, 42%, 42%, and 25%, respectively [sexes combined]. The percent of deaths at these respective dose levels for the sexes separately are 100%, 83%, 33%, and 17% for males and 100%, 0%, 50%, and 33% for females. The nominal concentrations were 0.42, 0.05, 0.11, and 0.12 mg/L. Signs of respiratory distress were observed at all dose levels, and lesions in the lungs were observed at necropsy. Because of an apparent inversion in the dose response relationship observed, the standard probit method [SAS CATMOD, 1985] could not be used to calculate the LC_{50} for the test material, according to the authors. The Thompson's Moving Average method was used to determine the LC_{50}. The LC_{50} was 0.21 mg/L [0.15-0.30 mg/L] for males and 0.34 mg/L [0.24-0.49 mg/L] for females. Five of the 6 males at the 0.20 mg/L [0.05 mg/L (nominal) concentration died, but none of the females in this group died. Mortality at the other dose levels was comparable between the sexes. The females at the 0.20 mg/L dose were affected by exposure as evidenced by slight to moderate rales and gasping [one female displayed rales through day 13], decreased body-weight gain in female survivors, and slight to moderate redness of the lungs at necropsy. The authors considered the apparent difference in mortality between the sexes at this dose level to have occurred by chance, and the LC_{50} was calculated for the combined sexes. The 4-hour LC_{50} was calculated to be 0.26 mg RH-287 Technical/L of air [95% confidence limits: 0.19-0.35 mg/L]. The MMAD determinations for RH-287 Technical resulted in values of 1.4, 2.1, 1.5, and 1.3 μm, with geometric standard deviations of 1.7, 1.9, 1.7, and 1.8 μm, respectively for the 0.23, 0.12, 0.46, and 0.20 mg/kg dose levels. The mean respirable fraction was $\approx$ 88%, 72%, 84%, and 85%, respectively.

TB II does not agree with the calculated LC_{50}, and the discrepancies between nominal and analytical concentrations raise questions as to the actual exposures attained. Since this test material is an obvious irritant, a repeat of the study is **not** recommended. All doses tested fall into Toxicity Category II. Given the death of 5 of the 6 males at the 0.2 mg/L [0.05 mg/L nominal] concentration, for the males, Toxicity Category I applies. The study is acceptable for determining the Toxicity Category for acute inhalation exposure.

D. CONCLUSION

Under the conditions of the study, exposure of rats to aerosol/vapor of RH-287 Technical at analytical dose levels of 0.12, 0.20, 0.23, and 0.46 mg/L [nominal concentrations: 0.11, 0.05, 0.12, and 0.42, respectively] for 4 hours [nose only] resulted in the death of all rats in both sexes at the highest dose level and of 5 of 6 males but none of the females at the 0.20 mg/L dose level. Based on the nominal concentrations, these dose levels represent the highest and lowest doses tested. Due to unexplained discrepancies between the nominal and

analytical concentrations, no LC_{50} can be calculated. It is concluded that the study is adequate for the determination of the Toxicity Category for acute inhalation exposure for RH-287 T Technical, and the study is classified Acceptable. Although this study does not satisfy the guideline requirement [81-3] for an acute inhalation study in rodents, no repeat study is recommended due to its irritant properties.

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011587

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 4/25/95
Tox. Branch II, Section II (7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 5/1/95
Tox. Branch II, Head Section II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90-Day Oral - rat

TOX. CHEM. NO.: 314B

MRID NO.: 434716-03

Shaughnessey No.: 128101

TEST MATERIAL: RH-287 Technical

SYNONYMS: 4,5-dichloro-2-n-octyl-3(2H) isothiazolone

STUDY NUMBER: Report # 93R-249; Protocol # 93P-249

SPONSOR: Rohm and Haas Company

TESTING FACILITY: Rohm and Haas Company Toxicology Department

TITLE OF REPORT: RH-287 Technical: Three-Month Dietary Toxicity Study in Rats

AUTHOR: DL Shuey, EJ Kaminski, and DM Gillette

REPORT ISSUED: September 21, 1994

QUALITY ASSURANCE: A quality assurance statement and a GLP Compliance Statement were provided.

EXECUTIVE SUMMARY: Under the conditions of the study, oral administration of RH-287 Technical via the diet to Crl:CD®BR rats [10/sex/group] for 13 weeks at dose levels of 0, 100 ppm [6.2 ♂/7.2 ♀ mg/kg], 500 ppm [32.5 ♂/36.7 ♀ mg/kg], 1000 ppm [60.7 ♂/74.7 ♀ mg/kg], and 4000 ppm [248.2 ♂/278.4 ♀ mg/kg] resulted in decreased body weight throughout the study at the high-dose level in both sexes {♂♂ 86%/♀♀ 80% of control at 13 weeks}, decreased overall body-weight gain {♂♂ 79%/♀♀ 77% (4000 ppm)/♀♀ 85% (1000 ppm) of control}, decreased food consumption at 4000 ppm for both sexes and at 1000 ppm in females initially, decreased water consumption at 4000 ppm, changes in several hematology/blood chemistry parameters consistent with chronic, low-level blood loss, and microscopic, treatment-related, changes in the forestomach {↑ mucosal erosion/ulceration, submucosal edema, acute inflammation, hyperplasia of the mucosal epithelium, and/or hyperkeratosis}. **The NOEL can be set at 500 ppm [32.5 ♂♂ /36.7 ♀♀ mg/kg/day], the LEL at 1000 ppm [60.7 ♂♂ /74.7 ♀♀ mg/kg/day], based on microscopic lesions in the forestomach in males and decreased body weight/gain and food consumption, decreased serum triglyceride levels, and microscopic lesions in the forestomach in females.**

This study is classified Core Minimum, and it satisfies the guideline requirement [82-1(a)] for a subchronic oral toxicity study in rodents.

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A. MATERIALS:

1. Test Compound: RH-287 Technical; Description: waxy, low-melting temperature solid; Batch #: Lot # 4086 [TD No. 93-115]; Purity: 98.8% 4,5-dichloro-2-n-octyl-3(2H)isothiazolone.

2. Test Animals: Species: rat; Strain: Crl:CD®BR; Age: ≈ 4 weeks old on receipt; Weight: ≈204-215 g ♂♂/≈152-159 g ♀♀ at week 0; Source: Charles River Laboratories, Kingston Facility, Kingston, NY.

3. Statistics: Body weight, weekly and cumulative body-weight changes, feed and water consumption, organ weights, hematology, blood chemistry, urinalysis, and WBC differentials: distribution of parameters inspected for normality and homogeneity of variance. For WBC differential data, the various cell type values were transformed using a square root function prior to analysis. Transformations were performed if necessary before utilizing analysis of variance [or covariance] to assess the presence or absence of an overall treatment effect. Group means were compared using least square means and when significant treatment effect was found, comparisons between control and appropriate treatment groups were calculated using Dunnett's test.

B. STUDY DESIGN

1. Methodology: Fifty males and 50 females [acclimated for ≈2 weeks] were selected from the original 55 rats/sex. One week prior to study initiation, the rats were weighed and given physical and ophthalmologic examinations. Those in good health were randomly assigned to a study group based on a computerized distribution according to body weight. There were five groups, each composed of 10 rats/sex, and each [except controls] was administered the test material [100, 500, 1000, or 4000 ppm] via the diet for 13 consecutive weeks. The controls received untreated diet. The dietary concentrations were selected based on the results from two previous oral studies [28-day study (89RC-1033) and a 2-week study (93P-228)]. The rats were provided with feed [PMI Certified Rodent Diet #5002M, previously known as Purina Certified Rodent Chow RP 5002M] and water ad libitum.

Dose preparation: The test material was placed in an oven [45-50°C] overnight prior to diet preparation. The appropriate amount of test material for each diet level was dissolved in acetone and mixed with feed to form a uniform premix; the acetone was evaporated off. The premix was added to additional untreated feed to bring to a total of 8 kg, and each diet concentration was stored in a bag [not further defined]. Diets were prepared weekly and stored frozen, according to Appendix Q. From week 1, samples from the top, middle, and bottom of the feed at each dose level were analyzed for homogeneity and to determine the accuracy of the preparations. Samples from

Week 1 were analyzed to determine stability of the diet at room temperature.

RESULTS

The diets appear to have been adequately mixed, and the concentrations attained were within 14% of the intended concentration for diets prepared during the first 4 weeks [Table 1]. With the exception of the low-dose level diet for week 12, the diets prepared and analyzed subsequently were within 20% of the intended concentration. The test material was stable in the feed, with >89% of the original active ingredient being found after 8 days of room temperature storage.

Table 1. Test Material Concentrations [% of intended concentration]

Week of Study/Dose	100 ppm	500 ppm	1000 ppm	4000 ppm
Week 1	87.2	93.4	93.8	95.1
top	87.8	91	94	97
middle	87.3	94	95	96
bottom	86.5	95	93	93
Week 2	90.9	99	98	96
Week 4	86.8	91	102	101
Week 8	81.8	90	87	93
Week 12	77.5	91	81	95

Test material intake was calculated weekly. The amount of test material ingested by each group is presented in Table 2.

Table 2. Dosage of Test Material Achieved [mg/kg/day]

Intake (mg/kg)/Dose (ppm)	100 ppm	500 ppm	1000 ppm	4000 ppm
males	6.2	32.5	60.7	248.2
females	7.2	36.7	74.7	278.4

Clinical Observations: Each rat was observed daily for signs of systemic toxicity or ill-health, and cage liners were inspected for abnormalities in urine and feces. A detailed physical examination of each rat was performed each week starting one week prior to initial dosing. The examination included evaluation of external structures, posture, gait, behavior, with gross abnormalities in respiration and body temperature being noted, and each rat was thoroughly palpated for surface and subcutaneous tissue masses and the oral cavity was inspected. Individual body weights and the quantity of feed and water consumed were determined weekly from one week prior to dosing. Compound intake was calculated for each rat as follows:

$$\frac{\text{Concentration in diet [ppm] x feed consumption [g/day]}}{\text{Body weight [g] at end of week}}$$

RESULTS

Survival and Clinical Observations

There were no treatment-related deaths. One control male was found dead during week 10, but no cause of death was determined. The only observation attributed to treatment was scant feces at the high-dose level [both sexes] during the first week of treatment.

Body Weight

From week 1 on, females at the 1000 ppm dose level and rats of both sexes at the 4000 ppm dose level displayed a significant decrease in body weight compared to the control groups. Cumulative body-weight change was significantly decreased in females at 1000 ppm from week 1 to 9 of dosing and in both sexes at 4000 ppm throughout the study, and the decrease was dose-related. Weekly body-weight gains varied widely among the groups throughout the study, with a significant overall body-weight decrease being observed in females at 1000 ppm and in both sexes at 4000 ppm [Table 4]. NOTE: The sharp decrease in body-weight gain in the low-dose females at week 13 does not appear to be due to either decreased food or water consumption.

Table 3. Mean Body Weight [% of control]

Sex/Dose (ppm)/Time♦	100	500	1000	4000
MALES				
-1	94	95	103	98
0	96	96	101	99
1	96	96	100	72*
2	97	96	99	70*
3	97	97	98	77*
4	97	97	96	80*
5	97	98	97	82*
6	97	97	96	83*
7	96	98	96	80*
8	96	98	95	83*
9	98	100	96	84*
10	98	98	96	85*
11	98	99	97	85*
12	98	100	97	86*
13	96	100	96	86*
FEMALES				
-1	101	100	102	99
0	99	96	99	96
1	98	94	94	85*
2	97	94	92*	87*
3	95	92	90*	85*
4	95	93	90*	84*
5	96	94	91*	83*
6	97	95	90*	88*
7	96	94	90*	83*
8	96	94	89*	83*
9	95	95	92*	82*
10	95	95	93	83*
11	96	96	94	83*
12	96	95	93	81*
13	94	94	92	80*

* $p<0.05$; ♦ week

Table 4. Mean Body-Weight Change [grams (% of control value)]

Interval (wk)/Group	0 ppm	100 ppm	500 ppm	1000 ppm	4000 ppm
MALES					
1	60.9	58.1	58.7	57.1	-12.9*
2	63.2	63.8	62.7	59.8	39.1*(62)
3	49.7	49.4	48.8	47.1	59.3*(119)
4	41.1	38.5	42.1	30.2	43.6
7	29.6	25.7	32.6	27.2	13.5(46)
9	17.5	24.0	25.0	20.5	23.7(135)
0 to 13	400.4	386.9	412.0	376.3(94)	318*(79)
FEMALES					
1	23.4	21.2(91)	18.3(78)	13.8*(59)	1.5*(6)
2	24.4	21.5(88)	23.2	18.4*(75)	24.7
3	22.0	18.4(84)	17.5(80)	15.4(70)	15.1(69)
4	15.9	15.3	16.0	15.8	12.3(77)
5	12.9	13.1	13.5	13.3	7.7(60)
6	7.3	9.2	9.5(130)	5.3(73)	9.7(133)
8	9.0	8.6	8.6	5.5(61)	7.9(88)
13	4.9	0.9(18)	3.5(71)	2.3(47)	3.8(78)
0 to 13	160.0	143.8(90)	148.8	136.5*(85)	106.9*(67)

* P<0.05;

Food and Water Consumption: Food consumption was decreased at the high-dose level for both sexes [p<0.05 was not always attained, especially in males] and during the first 3 weeks at the 1000 ppm dose level for females [Table 5].

Table 5. Mean Feed Consumption [% of control]

Sex/Dose (ppm)/Time♦	100	500	1000	4000
MALES				
0	97	98	99	98
1	96	97	95	41*
2	98	00	97	61*
3	98	103	100	87*
4	97	105	95	91*
7	96	104	95	85*
9	98	107	92	92
13	96	108	95	93
FEMALES				
0	96	96	97	94
1	93	93	88*	53*
2	93	94	88*	87*
3	92	91	89*	81*
4	91	91	91	79*
5	93	93	94	77*
7	97	97	95	82*
9	92	98	98	82*
13	95	95	97	85

* p<0.05; ♦ week

Water consumption was significantly decreased compared to the control value in the high-dose males during weeks 1 [55% of control] and 2 [68% of control]. High-dose females displayed decreased water consumption throughout the study, but statistical significance was attained only at week 13. In general, the control females displayed the highest water intake throughout the study. Since there were no water

consumption data collected prior to dose initiation, it is difficult to attribute the decrease at the high-dose level to treatment. The range of values for the controls was 208-296 grams and 160-190 grams for the high-dose females.

Table 6. Mean Water Consumption [% of control]

Sex/Dose (ppm)/Time♦	100	500	1000	4000
FEMALES				
0	91	89	100	88
1	85	89	95	75
2	78	78	90	73
3	88	89	106	74
4	86	91	98	71
5	89	82	97	60
13	101	83	86	61*

* $p<0.05$; ♦ week

3. Clinical Pathology

Blood was collected (following an overnight fast; via abdominal aorta, under pentabarbital anaesthesia) from all rats after 3 months of treatment. The CHECKED (X) parameters were evaluated.

Hematology

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count	X	Reticulocyte count
	Blood clotting measurements	X	Red cell morphology
	(Thromboplastin time)		
	(Activated partial thromboplastin time)		
	(Prothrombin time)		

RESULTS

The values of several parameters were statistically significantly different between control and high-dose rats [Table 6]. The incidence of altered red blood cell morphology was increased in both sexes at the high-dose level [Table 7]. It is to be noted that the RBC levels were increased at the high-dose level in males compared to the control value, while the values for HGB and HCT were decreased.

Table 6. Hematology Parameters

Interval/Group	0 ppm	100 ppm	500 ppm	1000 ppm	4000 ppm
MALES					
RBC [10^6/CMM]	8.81	9.04	8.86	8.88	9.50*(108)♦
HGB [G/DL]	16.2	16.4	16.0	16.3	13.8*(85)
HCT [%]	44.4	44.7	43.8	43.6	37.9*(85)
MCV [CM]	50.4	49.5	49.5	49.2	39.9*(79)
MCH [PG]	18.4	18.2	18.2	18.4	14.6*(79)
PLAT [1000/CMM]	1117	1146	1084	1104	1314*(118)
FEMALES					
RBC [10^6/CMM]	7.74	7.59	7.65	7.86	8.08(104)
HGB [G/DL]	15.8	15.7	15.6	15.5	15.3
HCT [%]	42.4	41.3	41.6	41.7	41.6
MCV [CM]	54.6	54.4	54.5	53.2	51.5*(94)
MCH [PG]	20.5	20.7	20.5	19.8	19.0*(93)
PLAT [1000/CMM]	1067	1046	1048	1046	1173(110)
WBC [1000/CMM]	5.4	5.2	5.8	5.5	4.2(78)

♦ (% of control); * p <0.05

Table 7. Incidence of RBC Morphology [# rats displaying finding]

Interval/Sex/Dose (ppm)	0	100	500	1000	4000
MALES n=	10	10	10	10	10
normal	9	10	10	10	1
anisocytosis	0	0	0	0	8
microcytosis	0	0	0	0	8
target cells	0	0	0	0	4
hypochromia	0	0	0	0	2
polychromia	0	0	0	0	0
FEMALES n=	10	9	10	10	10
normal	10	9	9	10	6
anisocytosis	0	0	0	0	4
microcytosis	0	0	0	0	4
target cells	0	0	0	0	0
hypochromia	0	0	0	0	0
polychromia	0	0	1	0	0

Blood Chemistry

Electrolytes:
- [X] Calcium
- [X] Chloride
- [ ] Magnesium
- [X] Phosphorous
- [X] Potassium
- [X] Sodium
- [ ] Iron

Enzymes
- [X] Alkaline phosphatase (ALK)
- [ ] Cholinesterase (ChE)
- [ ] Creatine kinase (CK)
- [ ] Lactate dehydrogenase (LAD)
- [X] Serum alanine aminotransferase
- [X] Serum aspartate aminotransferase
- [X] Gamma glutamyl transferase (GGT)
- [ ] Glutamate dehydrogenase (GLDH)
- [ ] Ornithine carbamyltransferase (OCT)
- [ ] Electrophoretic protein fractions

Other:
- [X] Albumin
- [X] Blood creatinine
- [X] Blood urea nitrogen
- [X] Cholesterol
- [X] Globulin
- [X] Glucose
- [ ] Phospholipids
- [X] Total bilirubin
- [X] Total Protein (TP)
- [X] Triglycerides
- [X] A/G ratio
- [ ] Triiodothyronine [T3]
- [ ] Thyroxine [T4]

RESULTS

Several statistically significant differences were observed in the monitored parameters, but the magnitude of the difference was small. Serum triglycerides were significantly decreased in females at 1000 ppm and in both sexes at 4000 ppm. Both sexes at the high-dose level also displayed significant decreases in total serum protein and globulin levels. Other differences observed in the clinical chemistry parameters are shown in Table 8.

Table 8. Clinical Chemistry Parameters

Interval/Group	0 ppm	100 ppm	500 ppm	1000 ppm	4000 ppm
MALES					
TRIG [G/DL]	90	61(68)♦	82(91)	79(88)	34*(38)
TP [G/DL]	6.1	6.1	6.1	6.0	5.7*(93)
GLOB [G/DL]	2.2	2.2	2.1	2.1	1.8*(82)
PHOS [MG/DL]	6.5	6.8	6.6	6.8	8.8*(135)
K [MMOL/L]	4.4	4.5	4.5	4.5	5.0*(116)
AG [ratio]	1.8	1.8	2.0(111)	2.0(111)	2.2*(122)
GOT [U/L]	98	109	123(126)	112(114)	138*(141)
BUN [MG/DL]	13.8	14.1	13.7	13.9	15.8(114)
FEMALES					
TRIG [G/DL]	68	48(71)	44(65)	40*(59)	32*(47)
TP [G/DL]	6.4	6.4	6.3	6.1	6.0*(94)
GLOB [G/DL]	1.9	1.9	1.8	1.7(89)	1.5*(79)
PHOS [MG/DL]	7.4	6.9	6.7	7.1	9.0(122)
K [MMOL/L]	4.2	4.3	4.2	4.3	5.0*(119)
AG [ratio]	2.5	2.5	2.6	2.7(108)	3.0*(120)
GOT [U/L]	90	121*(134)	98	108	100
BUN [MG/DL]	16.0	17.2(108)	15.9	17.1(107)	20.5*(128)

♦ (% of control); * p <0.05

4. Urinalysis: Urine samples [freshly-voided] were collected from all rats during week 13 [no indication of whether rats were fasted and deprived of water overnight]. The CHECKED (X) parameters were examined.

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

RESULTS

There were no treatment-related effects observed.

Ophthalmoscopy: Prior to study initiation [all rats] and at termination [survivors], indirect ophthalmoscopic examinations were performed [no other details provided].

RESULTS

There were no treatment-related ocular lesions observed.

7. Gross Pathology: All survivors were anesthetized, euthanized by exsanguination, and necropsied at sacrifice (following 13 weeks of treatment). The following organs were weighed: kidneys, liver, testes, adrenal glands, and brain.

RESULTS

There were no treatment-related gross pathology findings.

Organ weights: With the exception of the brain, all absolute organ weights were decreased and all relative organ weights were increased relative to the control. Absolute brain weight was comparable among the groups, and the relative brain weight at the high-dose level in both sexes was significantly increased [Table 9]. The differences observed can be attributed to the decreased body weight observed at termination.

Table 9. ABSOLUTE/RELATIVE ORGAN WEIGHT (grams)

Group Dose (ppm) Organ	0	100	500	1000	4000
MALES					
BODY WEIGHT	**575.0**	**554.6**	**581.2**	**555.6**	**494.1*(86)♦**
brain					
absolute	2.139	2.137	2.116	2.116	2.138
relative	0.375	0.387	0.367	0.383	0.439*(117)
FEMALES					
BODY WEIGHT	**292.4**	**277.7**	**279.2**	**273.0**	**237.9*(81)**
brain					
absolute	1.919	1.886	1.902	1.884	1.880
relative	0.663	0.685	0.687	0.706	0.799*(121)

* $p<0.05$; ♦ (% of control)

7. Histopathology: The following organs/tissues (CHECKED (X)) were preserved from all rats. Microscopic examinations were restricted to macroscopic abnormalities, lungs, liver, kidneys, glandular region of the stomach, and forestomach for all rats and all organs from all control and high-dose groups.

Digestive system		**Cardiovasc./Hemat.**		**Neurologic**	
	Tongue	X	Aorta	X	Brain
X	Salivary glands	X	Heart	X	Sciatic nerve
X	Esophagus	X	Bone marrow♥	X	Spinal cord✣
X	Stomach	X	Lymph nodes♦	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes/optic nerve
X	Jejunum	X	Thymus	**Glandular**	
X	Ileum	**Urogenital**		X	Adrenal gland
X	Cecum	X	Kidneys	X	Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland ♀♀
X	Rectum	X	Testes	X	Parathyroids
X	Liver	X	Epididymides	X	Thyroids
	Gall bladder	X	Prostate	**Other**	
X	Pancreas	X	Seminal vesicle	X	Bone/femur
Respiratory		X	Ovaries/Cervix	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung	X	Vagina	X	All gross lesions
	Nasal cavity	X	Harderian gland	X	Harderian gland
	Pharynx		Skull & ears	X	Coagulating gland
	Larynx	♦ mandibular & mesenteric; ♥ sternum & femur; ✣cervical, mid-thoracic, & lumbar			

RESULTS

Microscopically, treatment-related changes were observed in the forestomach [Table 10] in the 1000 ppm and 4000 ppm dose groups. At 1000 ppm, one male displayed minimal mucosal erosion and minimal acute inflammation of the mucosa/submucosa, and one female displayed minimal mucosal erosion with mild submucosal inflammation and moderate submucosal edema. All 4000 ppm rats displayed microscopic lesions of the forestomach. NOTE: In the text on page 29 of the report, it states that minimal hyperkeratosis was observed in 6/10 males and 5/10 females at the 4000 ppm dose level, but the Summary Table 4 [pages 261-262] and Individual Histopathology Table 5 [♂♂-page 268; ♀♀-page 292] in Appendix P list 7/10 males and 10/10 females with this finding. There were no other apparent treatment-related histopathological findings.

Table 10. Microscopic Findings [# with finding]

Lesion / Group / Dose	MALES					FEMALES				
	0	100	500	1000	4000	0	100	500	1000	4000
Forestomach N=	**10**	**10**	**10**	**10**	**10**	**9**	**9**	**10**	**8**	**9**
mucosal erosion/ulceration										
minimal	0	0	0	1	2	0	0	0	1	1
moderate	0	0	0	0	1	0	0	0	0	0
submucosal edema										
minimal	0	0	0	0	0	0	0	0	0	2
slight	0	0	0	0	1	0	0	0	0	1
moderate	0	0	0	0	3	0	0	0	1	0
inflammation, acute, mucosa/submucosa										
minimal	0	0	0	1	3	0	0	0	0	3
slight	0	0	0	0	1	0	0	0	1	0
hyperplasia, mucosal epithelium										
minimal	0	0	0	0	0	0	0	0	0	1
slight	0	0	0	0	4	0	0	0	0	4
hyperkeratosis										
minimal	0	0	0	0	7	0	0	0	0	10

DISCUSSION

All treated rats survived to study termination, and the only clinical sign of toxicity observed was scant feces at the high-dose level [both sexes]. Decreased body weight, body-weight gain, and food consumption were observed in females at the 1000 ppm dose level and in both sexes at the 4000 ppm dose level. Water consumption was reduced at the 4000 ppm dose level compared to the controls in both sexes. Altered red blood cell morphology [anisocytosis, microcytosis, target RBC (♂♂), and hypochromia (♂♂)] was observed in both sexes at the high-dose level, and several other hematology parameters [↓ MCV and MCH (♂♂ & ♀♀), ↑ RBC and platelets (♂♂), ↓ HBG and HCT (♂♂)] were observed at the 4000 ppm dose level that are consistent with chronic, low-level blood loss, which might be related to the gastric lesions observed. Decreased

triglycerides, serum protein, and globulin levels and increased potassium, AG ratio, and blood urea nitrogen values were observed at the 4000 ppm dose level in both sexes. Urinalysis and ophthalmoscopic findings were comparable among the groups for both sexes. Relative brain weight was increased in both sexes at the 4000 ppm dose level, but this was attributed to the differences in body weight at termination. The only treatment-related microscopic difference observed among the groups was found in the forestomach of the high-dose rats [both sexes]. All high-dose rats displayed microscopic lesions [mucosal erosion/ulceration, submucosal edema, acute inflammation, hyperplasia of the mucosal epithelium, and/or hyperkeratosis] in the forestomach in females.

CONCLUSION

Under the conditions of the study, oral administration of RH-287 Technical via the diet to Crl:CD®BR rats [10/sex/group] for 13 weeks at dose levels of 0, 100 ppm [6.2 ♂/7.2 ♀ mg/kg], 500 ppm [32.5 ♂/36.7 ♀ mg/kg], 1000 ppm [60.7 ♂/74.7 ♀ mg/kg], and 4000 ppm [248.2 ♂/278.4 ♀ mg/kg] resulted in decreased body weight throughout the study at the high-dose level in both sexes {♂♂ 86%/♀♀ 80% of control at 13 weeks}, decreased overall body-weight gain {♂♂ 79%/♀♀ 77% (4000 ppm)/♀♀ 85% (1000 ppm) of control}, decreased food consumption at 4000 ppm for both sexes and at 1000 ppm in females initially, decreased water consumption at 4000 ppm, changes in several hematology/blood chemistry parameters consistent with chronic, low-level blood loss, and microscopic, treatment-related, changes in the forestomach {↑ mucosal erosion/ulceration, submucosal edema, acute inflammation, hyperplasia of the mucosal epithelium, and/or hyperkeratosis}. **The NOEL can be set at 500 ppm [32.5 ♂♂ /36.7 ♀♀ mg/kg/day], the LEL at 1000 ppm [60.7 ♂♂ /74.7 ♀♀ mg/kg/day], based on microscopic lesions in the forestomach in males and decreased body weight/gain and food consumption, decreased serum triglyceride levels, and microscopic lesions in the forestomach in females.**

This study is classified Core Minimum, and it satisfies the guideline requirement [82-1(a)] for a subchronic oral toxicity study in rodents.

Table 11. Summary of Effects

Effect/ Sex/ Dose (ppm)	MALES				FEMALES			
	100	500	1000	4000	100	500	1000	4000
↓ body weight/gain	-	-	-	X	-	-	X	X
transient scant feces	-	-	-	X	-	-	-	X
↓ food & water consumption	-	-	-	X	-	-	X	X
↓ MCV & MCH	-	-	-	X	-	-	-	X
↑ RBC & platelet counts	-	-	-	X	-	-	-	-
↓ HGB & HCT	-	-	-	X	-	-	-	-
↑ serum GOT activity	-	-	-	X	-	-	-	-
↑ BUN	-	-	-	-	-	-	-	X
altered RBC morphology	-	-	-	X	-	-	-	X
↓ serum triglyceride levels	-	-	-	X	-	-	X	X
↓ serum protein & globulin levels	-	-	-	X	-	-	-	X
↑ serum P & K levels	-	-	-	X	-	-	-	X
↑ serum A/G ratio	-	-	-	X	-	-	-	X
↑ relative brain weight	-	-	-	X	-	-	-	X
microscopic lesions in forestomach	-	-	X	X	-	-	X	X

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Reviewed by: Linda L. Taylor, Ph.D Linda Lee Taylor 5/23/95
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Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 5/23/95
Section II Head, Tox. Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity rat TOX. CHEM. NO.: 314B

MRID NO.: 434716-04 SHAUGHNESSEY NO.: 128101

TEST MATERIAL: RH-287T Technical

SYNONYMS: 4,5-dichloro-2-n-octyl-3(2H) isothiazolone

STUDY NUMBER: Report # 93R-229; Protocol # 93P-229

SPONSOR: Rohm and Haas Company

TESTING FACILITY: Rohm and Haas Company Toxicology Department

TITLE OF REPORT: RH-287 Technical Oral (Gavage) Developmental Toxicity Study in Rats

AUTHOR: DL Shuey and AS Romanello

REPORT ISSUED: July 18, 1994

QUALITY ASSURANCE: A quality assurance statement and a Good Laboratory Practices Compliance Statement were provided.

EXECUTIVE SUMMARY: Under the conditions of the study, dose levels of 0, 10, 30, and 100 mg Kathon 287 Technical (98.8%)/kg of body weight/day administered to 25 pregnant female Crl:CD®BR rats per group during Days 6 through 15 of gestation [mated 1:1] resulted in (1) the death of 1/25 dams at the 100 mg/kg/day level, (2) decreased body-weight gain [73% of control] at the high-dose level during the dosing period with a concomitant decrease [88-90% of control] in food consumption, (3) decreased food consumption at the mid-dose level during the last week of dosing; (4) transient scant feces, soft feces, and/or diarrhea at the mid- and high-dose levels, and (5) an increased number of litters with fetuses with wavy ribs at the high-dose level. Pregnancy rate was comparable among the groups, and there were no statistically significant differences in the number of corpora lutea/dam, implantations/dam, live fetuses/dam, resorptions/dam, dead fetuses/dam, or in pre- or post-implantation losses, litter weight, net body-weight change in the dams, or fetal body weight (combined and per sex). There were no fetal external variations or developmental retardations, and there were no fetal soft tissue developmental retardations. Neither control group displayed fetal external malformations, fetal soft tissue malformations, and fetal soft tissue variations. There was an increase in the number of fetuses [21 vs 2] at the high-dose level with wavy ribs, along with an increase in the severity of the finding and a statistically significant increase in the number of high-dose litters [11 vs 1] with fetuses with wavy ribs compared to the concurrent control. There were no other statistically significant differences in the incidence of any fetal alterations that can be attributed to treatment. **The NOEL for**

maternal effects can be set at 10 mg/kg/day, the LEL at 30 mg/kg/day, based on decreased food consumption during the dosing period and transient scant feces, soft feces, and/or diarrhea. The NOEL for developmental toxicity can be set at 30 mg/kg/day, the LEL at 100 mg/kg/day, based on the increased number of litters with fetuses with wavy ribs.

This study is classified Core Minimum. This study satisfies the guideline requirement [83-3(a)] for a developmental toxicity study in the rat.

A. MATERIALS

1. Test Compound: RH-287 Technical; Description: tan, waxy solid; Batch #: Lot # 4086 TD# 93-115; Purity: 98.8%; Source: apparently supplied by Sponsor; Vehicle: corn oil [Lot # 42H0864; Sigma Chemical Co. St. Louis, MO.

2. Test Animals: Species: rat; Strain: Crl:CD®BR; Age: 55-63 days old on arrival; Weight: females: 177-224 g; Source: ♀♀ - Charles River Laboratories, Portage Facility, Portage, MI; ♂♂ - same strain and supplier [maintained at testing facility for breeding purposes] were used as sires.

3. Statistics: maternal body weights, body-weight gains, and food consumption: analysis of variance [ANOVA]; when one-way ANOVA was significant, Dunnett's test was used to detect significant differences between each treated group and concurrent control; implantations, corpora lutea, live fetuses, dead fetuses, resorptions, mean fetal weight/litter, and incidence of fetal alterations: Mann-Whitney U test; when more than 75% ties occurred [i.e., 75% of the litters are unaffected for a particular fetal parameter], then Fisher's exact test was used in place of the Mann-Whitney U test to detect significant differences between treated and concurrent control groups; incidence of pregnancy, adult necropsy findings, maternal deaths, and litters with total resorptions: Fisher's Exact test.

B. STUDY DESIGN

Groups of 25 pregnant female rats (acclimated for 7 days prior to study start) were treated orally via gavage once daily from days 6 to 15 of gestation at dose levels of 0, 10, 30, 100, or 300 mg RH-287 Technical/kg body weight [constant volume of 5 mL/kg]. NOTE: Due to deaths at the 300 mg/kg/day dose level and the early termination of this dose level for humane reasons, a second phase of the study was performed, which consisted of a control group and a 10 mg/kg/day dose group. The control females were administered the vehicle [warm corn oil] in the same volume as above. Prior to study initiation, females were mated [1 ♂ with 1 ♀] with the males and checked once daily for evidence of mating [presence of sperm plug(s)]. The day of mating was considered Day 0 of gestation. Each mated female was housed individually. Females were assigned to the groups using a randomized block design based on Day 0 of gestation body weights. After assignment to groups, the mean Day 0 body weight for each group was calculated and analyzed statistically. If significant differences were found, minimal switching of rats between groups was performed to reduce the differences prior to treatment. The dose administered each day was based on the most recent body weight of each animal. The dose levels were chosen based on the results of a range-finding study in non-pregnant female rats [Appendix 27 of report]. Prior to and during mating, the females received PMI Certified Rodent Diet #5002C [previously known as Purina Certified Rodent Chow #5002C]; after mating they received PMI Certified Rodent Diet #5002M [previously Purina Certified Rodent Chow #5002M]. Males received the former feed. Food was provided throughout the study on an ad libitum basis, and tap water was available ad libitum.

Dose Preparation: The test material was melted at ≈ 38-40°C and aliquoted into subsamples, which were remelted in an oven at the same temperature and used for one dose preparation. Dosing solutions were prepared every 4-5 days. The appropriate amount of test material was weighed out into a beaker and returned to the oven to prevent resolidification, and an appropriate amount of warm corn oil was added to the melted test material to attain the desired final volume. Each sample was mixed until the solution appeared homogeneous. Samples were refrigerated until use. Prior to dosing, the solutions were rewarmed to 38-40°C, continuously stirred using a magnetic stirbar and heated stirplate. Stability [following storage at room temperature or refrigeration], homogeneity [samples from top, middle, and bottom of first preparation at each dose level], and proximity to target concentrations [samples from top, middle, and bottom of first preparation at each dose level and from last and one other dose preparation] were assessed.

RESULTS

The test material was found to be stable under all tested conditions, and with the exception of the dose solution in the second phase of the study [87.5-89.1% of target], the % of target concentration found was ≥96.5% in the homogeneity and % of target concentration measurements.

C. Clinical Observations

Each dam was examined daily [Days 0-20 of gestation] in the morning for clinical signs and again in the afternoon on dosing days [Days 6-15]. Additionally, afternoon checks were performed on Days 0-5 and 16-19 of gestation for morbidity and mortality. Individual body weights were recorded in the morning on gestation days 0, 6, 8, 10, 13, 16, and 20. Feed jars were weighed on days 0, 6, 10, 16, and 20 of gestation.

D. Terminal Procedures

On Day 20 of gestation, all surviving dams were sacrificed via CO_2 asphyxiation and necropsied. The viscera were examined grossly immediately after sacrifice, and the stomach of each dam was inflated with buffered formalin and preserved for possible future examination. The carcasses were not saved.

E. Uterine/Implantation Data

The intact uterus of each dam was weighed, and corpora lutea for each ovary were counted. Implantation sites and early and late resorptions were counted. The uterus of apparently non-pregnant females was stained with ammonium sulfide to detect very early resorptions.

F. Fetal Data

All fetuses were examined, and the location in the uterus, sex, and condition were recorded. All live fetuses were weighed individually, and external alterations were recorded for each. Half of the live fetuses [distributed evenly throughout the uterus] of each litter were

examined for soft tissue alterations. All stunted, calculated stunted, and live fetuses with external malformations were examined for soft-tissue alterations also. Head alterations were recorded for those fetuses examined for soft tissue alterations. The heads were fixed and stored in Bouin's solution and examined using the technique of Barrow & Taylor. The fetuses were sacrificed by lethal i.p. injection of Nembutal®. All fetuses were examined for skeletal alterations [except the heads fixed in Bouin's solution were excluded]; stained with alizarin red S, following the method of Dawson. Dead fetuses were examined for external, soft tissue, and skeletal malformations.

RESULTS

1. Clinical Observations and Survival - Maternal

Nine dams at the high-dose level (300 mg/kg/day) died [no data were provided for this dose level, and it is not known when the dams began to die or how many doses were given], and the remaining dams exhibited signs of severe toxicity and extreme discomfort [no details were provided]. One additional female at the 100 mg/kg/day dose level was found dead on gestation Day 17. Scant feces was the only clinical sign observed, and blanching and thinning of the stomach wall, yellow fluid in the duodenum, and distention of the colon with gas were observed in this dam at necropsy. Since the death occurred after cessation of the dosing, the death could not be attributed to a dosing error and was considered treatment related. One death occurred at 30 mg/kg/day and was attributed to a dosing error. Treatment-related clinical signs included transient scant feces, soft feces, and/or diarrhea at the 30 mg/kg/day [21% incidence] and 100 mg/kg/day [72%] dose levels. One 10 mg/kg/day dam displayed scant feces for two days, but this occurred after the cessation of the dosing period and was not considered treatment-related. All other clinical findings were considered incidental.

2. Maternal Body Weight and Body-weight Gain

Body weight was comparable among the groups throughout the study, but body-weight gain was decreased at the mid- and high-dose levels during the dosing period, although the decrease at the mid-dose levels did not attain statistical significance. Compared to its control, the low-dose group displayed lower body-weight gains during the dosing period, but statistical significance was not attained. NOTE: The Day 0 body weight in the original study was between 241.1-242.5 grams; that in the add-on study was between 261.8-264.0 grams.

Table 1. Mean Body Weight [% of control]

Dose [mg/kg]/Day	10♦	30	100
0	99	100	100
6	99	99	100
8	100	99	99
10	99	98	99
13	99	97	96
16	98	98	97
20	98	99	99

♦ compared to its own control

Table 2. Body-weight Gains [grams (% of control)]

Dose (mg/kg)/Time Interval	0	0♦	10♦	30	100
0-6	23.2	31.4	29.9(95)♥	20.7(89)	23.4
6-8	2.6	0.0	2.6	2.7	-0.1
8-10	6.8	10.1	7.3(72)	4.6(68)	4.9(72)
10-13	11.4	16.9	16.9	9.3(82)	4.8*(42)
13-16	21.9	28.6	24.7(86)	23.5	22.5
6-16	42.7	55.6	51.4(92)	40.0(94)	31.1*(73)
16-20	61.7	72.9	73.0	64.2	67.0

* $p<0.05$; ♦ compared to its own different control; ♥ (% of control)

3. Food Consumption

The mid- and high-dose groups displayed lower food intakes during the dosing period, with statistically significant decreases being observed at the mid-dose level during Days 10-16 and at the high-dose level during Days 6-10 and 10-16 compared to the control value.

Table 3. Food Consumption [grams/animal/day]

Dose(mg/kg)/Interval(days)	0	0	10	30	100
0-6	20.1	21.5	21.8	19.4	21.0
6-10	16.8	17.9	17.8	15.8(94)♦	15.1*(90)
10-16	18.8	20.8	20.7	17.1*(91)	16.5*(88)
16-20	24.2	26.4	26.2	24.0	26.2*(107)

♦ % of control; * $p<0.05$

4. Gross Pathological Observations

There were no gross findings that could be attributed to treatment.

5. Maternal Observations

Pregnancy rate was comparable among the groups [96-100%], as were the number of corpora lutea, implantations, and live fetuses. There was no adverse effect observed on the number of resorptions, and both pre- and post-implantation losses were comparable among the groups. No litters were completely resorbed, and there were no dead fetuses. The Cesarean section observations are listed below.

Table 4: Cesarean Section observations

GROUP:	0 mg/kg	0 mg/kg♪	10 mg/kg♪	30 mg/kg	100 mg/kg
# Females mated	25	25	25	25	25
# Pregnant Females	24	24	25	25	25
Pregnancy Rate (%)	96	96	100	100	100
Maternal Wastage					
#Died	0	0	0	1	1
#Died/pregnant	0	0	0	1	1
#Died/Non pregnant	0	0	0	0	0
#Aborted♥	0	0	0	0	0
#Premature Delivery	0	0	0	0	0
# Females with 100% intrauterine deaths	0	0	0	0	0
# Females with live fetuses at necropsy (%)	24 (96)	24 (96)	25 (100)	24 (96)	24 (96)
Total # Corpora Lutea	421	439	438	403	416
Corpora Lutea/dam	17.5	18.3	17.5	16.8	17.3
Total # Implantations	348	395	404	359	344
Implantations/Dam	14.5	16.5	16.2	15.0	14.3
Total # Live Fetuses	337	383	380	347	333
Live Fetuses/Dam	14.0	16.0	15.2	14.5	13.9
% of implantations	96.8	97.0	94.1	96.7	96.8
Total # Resorptions	11	12	24	12	11
Early	11	12	23	11	10
Late	0	0	1	1	1
Resorptions/Doe	0.5	0.5	1.0	0.5	0.5
# Litters w/ resorptions (%)	9(38)	7(29)	13(52)	10(42)	9(38)
Total # Dead Fetuses	0	0	0	0	0
Postimplantation Loss(%)♦	3.1	3.0	6.2	3.6	3.2
Preimplantation Loss(%)	16.1	9.3	7.1	10.4	15.1
Litter Weight (gm)♦	48.1	59.9	56.9	49.0	47.2
Mean Fetal Weight (gm)	3.4	3.8	3.7	3.4	3.4
Mean Male Weight	3.5	3.9	3.9	3.5	3.5
Mean Female Weight	3.3	3.6	3.6	3.3	3.4
# Fetuses < 3 Grams	20	8	12	40	33
Sex Ratio (% Male)	52.3	46.1	53.5	48.3	49.3
Mean # Males	7.4	7.3	8.1	7.0	6.8
Mean # Females	6.7	8.6	7.1*	7.4	7.1
Gravid Uterus (gm)	76.2	92.0	89.1	79.1	74.5
Corrected Body Weight	293.1	331.9	327.1	286.9	290.5
Net Weight Change From Day 6	28.2	36.6	35.4	25.1 (89)♥	24.6 (87)♥

♪ 0 mg/kg/day and 10 mg/kg/day from 2nd phase of study; ♦ calculated by reviewer; ♥ (% of control); * p<0.052

6. Fetal Observations

None of the parameters monitored were adversely affected by treatment. The number of fetuses/litter was comparable among the groups, as were mean litter weight, mean fetal body weight (combined and per sex), and sex ratio. Although the number of females per litter at the 10 mg/kg/day dose level was statistically significantly decreased [7.1] compared to its concurrent control group [8.6], this was not considered treatment-related since there was no dose response and the number was comparable to the other groups in the other study.

There were no fetal external variations or developmental retardations, and there were no fetal soft tissue developmental retardations. Fetal external malformations, fetal soft tissue malformations, and fetal soft tissue variations were observed in the treated groups only [Table 5]. There was a statistically significant increase in the number of litters with fetuses with the skeletal variation wavy ribs at the high-dose level compared to the control and an increase in the number of high-dose fetuses with this variation, but statistical significance was not attained. The severity of the effects was increased also [Table 6].

Additionally, there was a dose-related increase in the number of litters with fetuses with developmental variations, which was statistically significant at the 30 and 100 mg/kg/day dose levels compared to the control value in that study [Table 7].

Table 5. Fetal Abnormalities - # (%)

Dose(mg/kg/day) # fetuses/# litters Defect	0		0		10		30		100	
	# fetuses	# litters	# fetuses	# litter	# fetuses	# litters	# fetuses	# litters	# fetuses	# litters
# fetuses/# litters examined										
external malformations	337	24	383	24	380	25	347	24	333	24
soft tissue malformations	176	24	197	24	197	25	182	24	168	23+
soft tissue variations	176	24	197	24	197	25	182	24	168	23+
EXTERNAL MALFORMATIONS										
exencephaly	0	0	0	0	0	0	1	1	0	0
agnathia	0	0	0	0	0	0	0	0	1	1
brachygnathia	0	0	0	0	0	0	1	1	0	0
anophthalmia, unilateral	0	0	0	0	0	0	1	1	0	0
microphthalmia, unilateral	0	0	0	0	1	1	1	1	0	0
Total fetal external malformations	0	0	0	0	1	1	3	3	1	1
SOFT TISSUE MALFORMATIONS										
situs inversus with dextrocardia	0	0	0	0	0	0	0	0	1	1
anophthalmia, left	0	0	0	0	0	0	1	1	0	0
microphthalmia	0	0	0	0	2	2	1	1	0	0
Total fetal soft tissue malformations	0	0	0	0	2	2	2	2	1	1
SOFT TISSUE VARIATIONS										
spleen, red foci	0	0	0	0	20	0	3	1	1	1
Total fetal soft tissue variations	0	0	0	0	0	0	3	1	1	1
SKELETAL MALFORMATIONS										
skull, exencephaly	0	0	0	0	0	0	1	1	0	0
shortened mandible	0	0	0	0	0	0	0	0	1	1
premaxilla, small	0	0	0	0	0	0	0	0	1	1
nasal & premaxilla bones fused	0	0	0	0	0	0	0	0	1	1
cervical atlas stunted & misshapen	0	0	0	0	0	0	1	1	0	0
cervical axis stunted & misshapen	0	0	0	0	0	0	1	1	0	0
cervical vertebrae arches fused across midline	0	0	0	0	0	0	1	1	0	0
Total fetal skeletal malformations	0	0	0	0	0	0	2	2	1	1
SKELETAL VARIATIONS										
sternebra: asymmetrical	1	1	1	1	1	1	1	1	1	1
rib: extra ossification site [lumbar # 1]	4	2	16	10	11	7	0	0	3	3
rib: rudimentary : thoracic # 13	3	2	2	2	0	0	20	11*	6	5
rib: ossification site: thoracic # 13	0	0	0	0	0	0	8	5	0	0
wavy ribs	2	1	1	1	1	1	1	1	21	11*
Total fetal skeletal variations	10	6	20	11	13	8	27	13	30	16*

Table 5. Fetal Abnormalities - # (%)

Dose(mg/kg/day) # fetuses/# litters Defect	0		0		10		30		100	
	# fetuses	# litters	# fetuses	# litter	# fetuses	# litters	# fetuses	# litters	# fetuses	# litters
SKELETAL DEVELOPMENTAL RETARDATIONS										
skull, hyoid, partially ossified	7	6	37	14	21	11	10	6	14	8
skull, hyoid, unossified	29	13	25	13	34	16	32	13	41	16
skull, parietal, partially ossified	0	0	0	0	0	0	1	1	3	2
skull, interparietal, partially ossified	0	0	0	0	0	0	1	1	3	1
skull, supraoccipital, partially ossified	1	1	1	1	1	1	1	1	7	5
skull, frontal, partially ossified	0	0	0	0	0	0	1	1	0	0
cervical vertebrae, arches partially ossified	0	0	0	0	0	0	1	1	0	0
thoracic vertebrae, centrum dumbbelled	26	13	3	3	8	7	24	14	30	13
thoracic vertebrae, centrum bipartite	3	3	4	3	3	2	5	5	9	7
thoracic vertebrae, centrum dumbbelled & asymmetrically ossified	0	0	1	1	1	1	0	0	0	0
thoracic vertebrae centrum unossified	1	1	0	0	1	1	3	3	5	3
thoracic vertebrae, arches unossified	0	0	0	0	0	0	0	0	1	1
thoracic vertebrae, centrum partially ossified	3	2	0	0	1	1	1	1	2	2
thoracic vertebrae, centrum unilaterally ossified	0	0	0	0	0	0	2	1	0	0
lumbar vertebra, arches unossified	0	0	0	0	1	1	0	0	0	0
lumbar vertebrae, arches partially ossified	0	0	1	1	1	1	1	1	2	2
lumbar vertebra, centrum dumbbelled	1	1	0	0	0	0	1	1	0	0
sacral vertebra arches unossified	5	5	8	4	7	5	18	7	5	2
sacral vertebra arches partially unossified	42	17	41	14	33	14	59	20	59	18
sacral vertebra, centrum unossified	0	0	0	0	1	1	0	0	0	0
sternebra, partially ossified	193	24	274	24	268	25	212	24	195	24
sternebra unossified	160	24	142	22	152	23	164	23	178	23
sternebra, bipartite	1	1	0	0	0	0	1	1	2	2
rib, partially ossified	0	0	1	1	0	0	2	2	3	3
pelvis, pubis, partially ossified	2	1	28	10	14	7	18	12*	8	6
pelvis, pubis, unossified	0	0	8	2	4	3	3	2	5	1
pelvis, ischium partially ossified	0	0	9	8	1	1*	6	4	6	4
pelvis, ischium unossified	0	0	0	0	1	1	0	0	3	1
Total fetal skeletal developmental retardations	307	24	353	24	355	25	316	24	306	24

* $p<0.05$; ♦ data for 1 litter inadvertently not entered into computer

Table 6. Severity of Wavy Ribs

Severity Incidence Group	0		0		10		30		100	
	# fetuses	# litters	# fetuses	# litters	# fetuses	# litters	# fetuses	# litters	# fetuses	# litters
mild	0	0	0	0	0	0	0	0	4	4
moderate	2	1	1	1	1	1	1	1	9	6
severe	0	0	0	0	0	0	0	0	8	6
TOTAL	2	1	1	1	1	1	1	1	21	11

Table 7. Total Fetal Alterations

Defect/Group [mg/kg/day]	0	0	10	30	100
MALFORMATIONS					
FETAL INCIDENCE	0	0	2	4	2
LITTER INCIDENCE	0	0	2	4	2
mean litter %	0	0	0.55	1.26	0.56
VARIATIONS					
Developmental					
FETAL INCIDENCE	10	20	13	30	31
LITTER INCIDENCE	6	13	8	14*	17*
mean litter %	2.88	5.06	3.64	8.61	9.62
Retardations					
FETAL INCIDENCE	307	353	355	316	306
LITTER INCIDENCE	24	24	25	24	24
mean litter %	91.43	91.92	92.99	90.89	91.85
Total Variations					
FETAL INCIDENCE	307	355	357	317	307
LITTER INCIDENCE	24	24	25	24	24
mean litter %	91.43	92.46	93.61	91.12	92.17

D. Discussion

At the original high-dose level [300 mg/kg/day], 9 of 25 rats died during the dosing period, and this dose group was terminated and not reported. One pregnant dam at the 100 mg/kg/day dose level [considered the high dose] died on test, and one 30 mg/kg/day rat died due to a dosing error. There was a reduction in food consumption and body-weight gain during the dosing period at the 100 mg/kg/day dose level and a slight decrease in food consumption at the 30 mg/kg/day dose level during the second half of the dosing period. The high-dose dams displayed a significant increased food consumption during the Day 16-20 time interval compared to the control. The only clinical signs of toxicity observed were transient scant feces, soft feces, and/or diarrhea at the 30 mg/kg/day [21%] and 100 mg/kg/day [72%] dose levels. The pregnancy rate was comparable among the groups, and there were no significant differences among the groups in any of the Cesarean parameters measured. There was a slight decrease in the net weight gain from day 6 of gestation in the mid- [89%] and high-dose [87%] dams compared to the control value, but statistical significance was not attained. Litter and fetal weights were comparable among the groups, and the live fetuses were found to be normally developed at delivery. There was an increase in the number of fetuses [21 vs 2] at the high-dose level with wavy ribs, along with an increase in the severity of the finding and a statistically significant increase in the number of high-dose litters [11 vs 1] with fetuses with wavy ribs compared to the concurrent control. Historical control data were not provided.

Additionally, the total number of fetal skeletal variations was significantly increased at the high-dose level. Overall, there was a dose-related, statistically significant, increase in the number of litters with fetuses with developmental variations at the 30 and 100 mg/kg/day dose level compared to the concurrent control group. At the 30 mg/kg/day dose level, this increase was largely due to the increase in the number of litters with fetuses with rudimentary 13th thoracic ribs. At the high-dose level, the increase was due mainly to the increased incidence of wavy ribs.

D. CONCLUSION

Under the conditions of the study, dose levels of 0, 10, 30, and 100 mg Kathon 287 Technical/kg of body weight/day administered to 25 pregnant females per group during Days 6 through 15 of gestation [mated 1:1] resulted in (1) the death of 1/25 dams at the 100 mg/kg/day level, (2) decreased body-weight gain [73% of control] at the high-dose level during the dosing period with a concomitant decrease [88-90% of control] in food consumption, (3) decreased food consumption at the mid-dose level during the last week of dosing; (4) transient scant feces, soft feces, and/or diarrhea at the mid- and high-dose levels, and (5) an increased number of litters with fetuses with wavy ribs at the high-dose level. Pregnancy rate was comparable among the groups, and there were no statistically significant differences in the number of corpora lutea/dam, implantations/dam, live fetuses/dam, resorptions/dam, dead fetuses/dam, or in pre- or post-implantation losses, litter weight, net body-weight change in the dams, or fetal body weight (combined and per sex). There were no fetal external variations or developmental retardations, and there were no fetal soft tissue developmental retardations. Neither control group displayed fetal external malformations, fetal soft tissue malformations, and fetal soft tissue variations. There was an increase in the number of fetuses [21 vs 2] at the high-dose level with wavy ribs, along with an increase in the severity of the finding and a statistically significant increase in the number of high-dose litters [11 vs 1] with fetuses with wavy ribs compared to the concurrent control. There were no other statistically significant differences in the incidence of any fetal alterations that can be attributed to treatment. **The NOEL for maternal effects can be set at 10 mg/kg/day, the LEL at 30 mg/kg/day, based on decreased food consumption during the dosing period and transient scant feces, soft feces, and/or diarrhea. The NOEL for developmental toxicity can be set at 30 mg/kg/day, the LEL at 100 mg/kg/day, based on the increased number of litters with fetuses with wavy ribs.**

This study is classified Core Minimum. This study satisfies the guideline requirement [83-3(a)] for a developmental toxicity study in the rat.

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 6/8/95
Section II, Tox. Branch II (7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 6/12/95
Section II Head, Tox. Branch II (7509C)

DATA EVALUATION REPORT

011587

STUDY TYPE: metabolism TOX. CHEM. NO.: 314B

MRID NO.: 434716-09 PC Code: 128101

TEST MATERIAL: RH-287 Technical

SYNONYMS: Kathon 287T Industrial Microbicide; XB3 Technical HQ(RH-287)

CHEMICAL: 4,5-dichloro-2-n-octyl-3-(2H)-isothiazolone

STUDY NUMBER: Report # 92R-073; Protocol # 92P-073

SPONSOR: Rohm and Haas Company

TESTING FACILITY: Rohm and Haas Company Toxicology Department

TITLE OF REPORT: ^{14}C-RH-287: Pharmacokinetic Study in Rats

AUTHORS: LJ DiDonato and GA Hazleton

REPORT ISSUED: May 25, 1994

QUALITY ASSURANCE: A Quality Assurance statement and a Good Laboratory Practice Compliance statement were provided.

EXECUTIVE SUMMARY: Rats [Crl:CD®BR; 4 or 5/sex/group] were administered ^{14}C-RH-287 [4,5-labeled] orally single low [20 mg/kg], single high [250 mg/kg], or 14 consecutive unlabeleled low [20 mg/kg/day] followed by a single ^{14}C low [20 mg/kg] doses of RH-287 Technical. Following the single exposures, essentially all of the dose was excreted in the feces and urine, with >81% {**feces**: ♂♂ ≈74% [low]/≈81% [high]; ♀♀ ≈77% [low]/≈71% [high]; **urine**: ♂♂ ≈13% [low]/≈10% [high]; ♀♀ ≈16% [low]/≈10% [high]} being eliminated within 2 days. No sex difference was observed. Similarly following a 14-day repeated 20 mg/kg/day dose, >60% [**feces**: ♂♂ ≈53%/♀♀ ≈43%; **urine**: ♂♂ ≈ 17%/♀♀ ≈ 17%] of a single 20 mg/kg ^{14}C dose was eliminated within 2 days. Radiolabel was distributed to all tissues measured. The concentration of radiolabel remaining in the tissues at 96 hours was dose-related and represented ≈ 0.3% ♂♂/<0.5% ♀♀ of the administered dose. At 4 days post dose, the highest percentage of the dose was displayed in the liver, kidney, and stomach. The overall recovery of radiolabel following single-dose exposure was 90%-96%. Overall recovery following repeated dosing was lower [64%-74%], but this was attributed to differences in fecal homogenate preparation. Pretreatment for 14 days did not alter the elimination of the radiolabel. It does not appear that RH-287 Technical would bioaccumulate significantly.

Classification: Acceptable. The identification and characterization of

the metabolites will be reported in a separate report as a supplement to this report. This study does not satisfy the guideline requirement (85-1) for a metabolism study, but with the submission of an acceptable metabolite identification report, this data requirement will be satisfied.

A. MATERIAL

1. Test Compound: **Labeled compound** - ^{14}C-RH-287 (4,5 labeled); Physical Description: not provided; Source: presumably the Sponsor; Batch #: TD-92-072, Lot #: 630.0108 and TD-93-133, Lot # 726.0102; Purity: not provided. Specific Activity: 55.39 µCi/mg (Lot # 630.0108) and 21.6 µCi/mg (Lot # 726.0102). **Unlabeled compound** - RH-287; Physical Description: not provided. Batch #: TD-92-208 and TD-93-132, Lot #: 12-SS-51 and TD-93-063, Lot #: 4062-7; Purity: Lot #: 12-SS-51 [99.8%], Lot #: 4062-7 [99.7%].

Structure: No structure was provided.

2. Test Animals: Species: rat; Strain: Crl:CD®BR; Age: not stated; Weight: range: ♂♂ 258.1-493.6 g/♀♀ 212.7-344.9; Source: Charles River Breeding Kingston, Stone Ridge, NY.

B. STUDY DESIGN

1. Methodology: There were 14 groups [4 or 5/sex] in this study (described below), but there was no information in the final report as to how the animals were selected for the groups. The protocol indicates that only rats whose body weights were within 2 standard deviations of the mean body were selected for use, and a random assignment to groups by use of the LSCPROD computer system was performed. The rats in Groups A, B, E, F, G, H, K, and L were housed individually in Nalgene® plastic metabolism cages [Nalge Co. Rochester, NY] that were equipped for the collection and separation of urine and feces. Rats in Groups C, D, I, and J were individually housed in metal hanging cages for blood collection during the study and those in Groups M and N were housed similarly during the oral gavage pretreatment period and placed in metabolism cages for the "pulse" ^{14}C [according to the protocol]. Following an acclimation period in their respective cages of at least 3 days, the radiolabeled test material [see Table 1 below] was administered orally via gavage [5 mL/kg] to all groups except Groups M and N who received nonradiolabeled RH-287 Technical orally via gavage [5 mL/kg] at 20 mg/kg/day for 2 weeks prior to receiving a "pulse" dose [5 mg/kg] of radiolabeled RH-287 Technical at 20 mg/kg.

The animals were provided with Purina® Certified Rodent Lab Chow 5002(M) and tap water via water bottles [metabolism cages] or automatic watering [metal cages] ad libitum. All rats were observed daily for any clinical signs throughout the study. Body weights were recorded at randomization, prior to dosing, and at termination [Groups A, B, G, H, M, and N only]. Body weight and food consumption of Group M and N rats was measured weekly beginning at initiation of nonradiolabel dosing.

Samples of urine and feces from Groups A, B, G, H, M, and N were submitted for ^{14}C-metabolite analysis and are to be reported in a separate report as a supplement to the current report.

Table 1. Study Design

Group	Sex	Dose [mg ai/kg ^{14}C♣	n	Parameters Monitored♥
A	M	20	5	E [0 hrs/Days 1, 2, 3, 4]/K
B	F	20	5	E [0 hrs/Days 1, 2, 3, 4]/K
C	M	20	4	B [hrs 1, 3, 6, 10/Days 1, 2, 3, 4]/K*
D	F	20	4	B [hrs 1, 3, 6, 10/Days 1, 2, 3, 4]/K*
E	M	20	4	E [hrs 0, 6]/K^c [6 hrs]
F	F	20	4	E [hrs 0, 6]/K^c [6 hrs]
G	M	250	5	E [0 hrs/Days 1, 2, 3, 4]/K
H	F	250	5	E [0 hrs/Days 1, 2, 3, 4]/K
I	M	250	4	B [hrs 1, 3, 6, 10/Days 1, 2, 3, 4]/K*
J	F	250	4	B [hrs 1, 3, 6, 10/Days 1, 2, 3, 4]/K*
K	M	250	4	E [0 hrs/Day 1]/K^c [Day 1]
L	F	250	4	E [0 hrs/Day 1]/K^c [Day 1]
M	M	20♦	5	E [0 hrs/Days 1, 2, 3, 4]/K
N	F	20♦	5	E [0 hrs/Days 1, 2, 3, 4]/K

♣ single oral dose; ♦ rats received unlabeled RH-287 Technical [20 mg/kg/day] for two weeks prior to a single ^{14}C-RH-287 Technical dose; ♥ E: urine, urine funnel wash, and feces collected over dry ice of ^{14}C analysis; remaining excreta samples were stored frozen for ^{14}C-metabolite analysis; B: whole blood and plasma collected; K: killed and whole blood, liver, fat, kidney, bone marrow, heart, lungs, brain, testes [♂♂], ovaries [♀♀], muscle, spleen, adrenals, thyroids, and remaining carcass collected for ^{14}C-analysis; remaining samples stored frozen for possible metabolite analysis; K*: killed and total carcass stored frozen; K^c: killed at peak ^{14}C-plasma concentrations and tissues listed for K were analyzed [assumed by reviewer; as indicated in Protocol, Appendix C].

NOTE: In the Protocol [page 095; Protocol Amendment No. 1]], it states that Groups A, B, G, H, M, and N are supposed to have K* at day 4, and Groups C, D, I, and J are supposed to have K. The table on page 014 of the final report has not incorporated these amendment changes. Additionally, the Legend in the final report table does not define K^c.

2. Dose preparation: The dose solution for the low- and pulse-dose groups [Groups A, B, C, D, E, F, M, N] was prepared by dissolving 1 part ^{14}C-RH-287 Technical [TD 92-072; TD 93-133] and 39 parts nonradiolabeled RH-287 Technical [TD 92-208; TD 93-132] in olive oil to obtain a 4 mg/mL solution of RH-287 Technical. The dose solution of the high-dose groups [Groups G, H, I, J, K, L] was prepared by dissolving 1 part ^{14}C-RH-287 Technical [TD 92-072; TD 93-133] and 199 parts nonradiolabeled RH-287 Technical [TD 92-208; TD 93-132] in acetone. Subsequently, the acetone was evaporated under nitrogen, and the mixture was dissolved in olive oil to obtain a 50 mg/mL solution. All ^{14}C-dose solutions were prepared fresh on the day of dosing, and the solution for the pretreatment phase was prepared fresh at the beginning of the dosing period and stored at room temperature during the 2-week phase of the study. Both the high- and low-dose solutions were warmed in a water bath to 40°C and stirred during dosing. The 4 and 50 mg/mL dose solutions were used to dose the 20 and 250 mg/kg dose groups, respectively. The protocol states that aliquots of each dose solution would be taken with the same syringe and in the same manner as the actual dose for Liquid Scintillation Counting [LSC] to determine the activity of the ^{14}C dose solution and the total amount of ^{14}C-RH-287 Technical administered to each animal. Additionally, samples of nonradiolabeled test material suspensions were to be taken and samples submitted for analysis of active ingredient. Samples taken at the time of preparation and at the end of both week 1

and 2 were to be assessed for stability and an assessment of dose attained.

RESULTS

The dosing solutions analyzed [only low dose (4000 ppm or 4 mg/mL) data provided] were reported to contain 100% of the target concentration [range:98.6-101.5% of target]. Stability data were not provided. In Appendix G [Protocol Deviation 1], it is stated that samples were not taken at the end of each week; therefore, stability analysis was not possible. However, it is stated that subsequent analysis of the dosing solution found it to be 100% of the target value.

Sample collection: Urine and feces were collected [over dry ice] from all rats in Groups A, B, G, H, M, and N at intervals up to 4 days after dosing [0 hours and on days 1, 2, 3, and 4] and analyzed for ^{14}C-label. All rats in these groups were sacrificed [CO_2 anesthesia and exsanguination] after 4 days and whole blood [via abdominal aorta], liver, fat, kidney, bone marrow, heart, lungs, brain, testes [♂♂], ovaries [♀♀], muscle, spleen, adrenals, and thyroids were removed and analyzed for ^{14}C-label. As much blood as possible was collected from each rat and weighed, and an aliquot of the blood was weighed and analyzed for ^{14}C-label by combustion and LSC. The remaining blood was centrifuged to separate the plasma. An aliquot of plasma was analyzed for radiolabel by LSC, and the remaining plasma was stored frozen for possible ^{14}C-metabolite analysis. The above-listed tissues were weighed and portions analyzed for radiolabel by combustion and LSC. The remaining portions were stored frozen for possible ^{14}C-metabolite analysis. Additionally, the remaining carcass was collected for ^{14}C-analysis. Whole blood [via orbital sinus puncture after light ether anesthesia] and plasma [from whole blood centrifuged at 3000 x g] were collected from all rats in Groups C, D, I, and J and analyzed [whole blood by combustion and LSC; plasma not specified] for ^{14}C-label. At terminal sacrifice of these rats, whole blood and plasma were collected [abdominal aorta] and analyzed. The remaining carcass of each was stored frozen. The kinetics of elimination of ^{14}C label from the whole blood and plasma were determined by RSTRIP® [Micromath Scientific Software]. The urine and feces of all rats in Groups E and F, and K and L were collected at 6 and 24 hours post dose, respectively. The rats in these groups were killed when plasma and whole blood ^{14}C concentrations were at peak levels post dose, as determined from a previous ^{14}C-RH-287 range-finding study [92P-156] and Groups C, D, I, and J of the current study. Tissues [listed above for Groups A, B, G, H, M, and N] and the remaining carcasses were collected and analyzed for ^{14}C-label. NOTE: The final report did not provide many details, and the protocol had to be consulted for details.

Sample analyses: **Urine** The volumes of urine and urine funnel wash collections were recorded and aliquots of the undiluted urine were analyzed for radiolabel by liquid scintillation counting [LSC]. The remaining samples were stored frozen prior to ^{14}C-

metabolite analysis. **Feces** The weight of the feces was determined, and fecal homogenates for Groups A, B, E, F, G, H, K, and L were prepared by making a 25% homogenate in water of each sample, which was refrigerated for 1-2 days after which it was thawed [?] and a measured volume of 75% methanol [MeOH] in water was added to each homogenate to achieve a final MeOH concentration in the homogenate of 25%. Subsequently, aliquots of the homogenates were analyzed for radiolabel by combustion and LSC. The remaining fecal homogenates were stored frozen prior to ^{14}C-metabolite analysis. The fecal matter from Groups M and N was homogenized in an aqueous 75% MeOH solution, and these samples were analyzed and stored as described above. **Carcass** The remaining carcasses were frozen, chopped into sections, and homogenized with dry ice, which was sublimed and the remaining frozen powder/homogenates were weighed, aliquoted, and analyzed for radiolabel by combustion and LSC.

RESULTS

Recovery of radioactivity

The total recovery for each group was reported as follows:

Table 2. Overall Radioactivity Recovery (% of dose)

LOW DOSE		REPEAT DOSE		HIGH DOSE		LOW DOSE [6 HR]		HIGH DOSE [24 HR]	
Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
90	95	74	64	96	93	96	76	70	52

NOTE: It was stated that the low recovery in the repeated dose group could be attributed to the different methods used to prepare the fecal homogenates between the single low and repeated low groups. The low recoveries observed in the groups killed at 6 and 24 hours were due to loss of unabsorbed test material that would have been in the stomachs, which were inadvertently discarded.

Elimination

Following both the single low and the single high doses [both sexes], the majority [81-93% of the dose] of the radiolabel was eliminated within two days. Similarly following repeated dosing, 60-70% of the "pulse" dose was eliminated within 2 days. The primary route of elimination was via the feces for both sexes following oral administration of (1) a single high dose [day 4: 81-85% of dose], (2) a single low dose [day 4: 75-78% of dose, and (3) a single low dose after a 2-week repeated low dosing [day 4: 44-54% of the dose] regimen. NOTE: Text of report listed the amount recovered in the single low and single high dose groups as 78-85%. The numbers above reflect the range based on the individual values listed in Table 1 of the report.

Feces: The majority of the radioactivity excreted in the feces was excreted by both sexes within 24 hours post dose following low-dose exposure and within 2 days following both the high-dose

and repeated/pulse dose exposures [Table 3]. Urine: The majority of the radioactivity excreted in the urine was excreted by both sexes within 24 hours post dose following all dosing regimens [Table 3].

Table 3. Cumulative Excretion via the Urine and Feces (% Dose)

Regimen / Sex/Time / Excreta	LOW DOSE		HIGH DOSE		REPEATED DOSE	
	Males	Females	Males	Females	Males	Females
URINE						
Day 1	12.35	14.56	7.09	7.01	15.25	15.31
Day 2	13.27	16.15	9.67	10.31	17.06	16.84
Day 3	13.51	16.39	9.92	10.80	17.43	17.13
Day 4	13.69	16.57	10.05	11.01	17.59	17.28
FECES						
Day 1	68.74	61.77	41.18	33.93	35.57	29.70
Day 2	74.61	76.80	80.65	70.73	52.70	43.20
Day 3	75.06	77.47	84.11	80.16	54.15	44.06
Day 4	75.25	77.65	84.96	81.20	54.44	44.39
TOTAL						
Day 1	81.09	76.33	48.27	40.94	50.82	45.01
Day 2	87.88	92.95	90.32	81.03	69.75	60.04
Day 3	88.57	93.86	94.03	90.96	71.58	61.19
Day 4	88.93	94.21	95.01	92.21	72.03	61.67

Concentration of Radiolabel in the Blood and Tissues/Organs

Whole Blood: **Single low** - The concentration of radiolabel in the whole blood reached a peak between 10 and 24 hours, declining thereafter following the single low dose. The peak concentrations were 1.5 ppm for males and 1.2 ppm for females. The elimination of the radiolabel from whole blood was monophasic [Figure 7, copy appended]. The halflives of elimination were reported as 15.9 hours for males and 26.6 hours for females. At 4 days, radiolabel concentration in whole blood was 0.2 ppm. **Single high** - Peak concentrations [5.5 ppm for males and 14.8 ppm for females] of radiolabel were observed at 24 hours, with the concentrations declining thereafter [Figure 8, copy appended]. The elimination from whole blood was monophasic, and the halflives were reported as 25.0 hours for males and 48.4 hours for females. At 4 days, radiolabel concentration in whole blood was 3.3 ppm. It is stated [page 022 of report] that ^{14}C-concentrations in whole blood [0.2 ppm] of the repeat dose group [20 mg/kg] were comparable to those in whole blood [0.2 ppm] of the single low-dose [20 mg/kg] group, which suggests that predosing did not affect the rates of elimination of radiolabel. There were no data provided for the repeat dose group with respect to whole blood [except as % of administered dose] values.

Plasma: **Single low** - Peak concentrations [0.9 ppm for males and 1.2 ppm for females] were attained earlier [6 to 10 hours] in plasma than in whole blood [Figure 9, copy appended]. The halflives of elimination of radiolabel were 16.1 hours for males and 20.5 hours for females. The concentration at 4 days was 0.1 ppm. **Single high** - Peak concentrations [4.6 ppm for males and 8.6 ppm for females] were observed at 24 hours, and the halflives of radiolabel elimination were 19.4 hours in males and 25.0 hours in

females. The concentration at 4 days was 1.1 ppm. NOTE: No discussion was provided as to the methods used in calculating the halflives. Additionally, no data were provided on plasma values for the repeat dose group.

Table 4. Whole Blood/Plasma ^{14}C Concentration [µg equivalents/g whole blood or /mL plasma]

Regimen Sex Tissue/Hours	LOW DOSE		HIGH DOSE	
	Males	Females	Males	Females
Whole Blood				
1	0.5317	0.5588	3.7837	4.5542
3	0.7356	0.8109	3.0696	2.6997
6	0.8875	1.0820	3.5475	5.3885
10	0.8853	1.1627	5.3383	7.2434
24	1.4980	0.9738	5.4811	14.7930
48	0.3261	0.3454	3.5699	5.5101
72	0.2470	0.3633	2.7655	4.2892
96	0.2482	0.1980	2.0226	3.2984
Plasma				
1	0.6442	0.5797	2.3241	2.2513
3	0.7569	0.8851	1.9569	2.2418
6	0.8239	1.2192	2.4925	3.3238
10	0.8545	1.0099	3.5269	4.3048
24	0.3393	0.4812	4.6340	8.5612
48	0.1738	0.2478	2.0983	3.1925
72	0.1221	0.1942	1.2863	1.7015
96	0.0861	0.1226	0.8233	1.0895

Tissues/Organs: The levels of radioactivity remaining in the tissues at study termination [4 days following dosing or 6 and 24 hours post dose] are shown below (Table 5). At 4 days, 0.3 to 0.5 % of the administered dose was found in the tissues in the single and repeated low dose groups, and 0.2 to 0.4% of the administered dose was observed in the tissues in the single high-dose groups. The highest concentrations of radiolabel were observed (1) in the liver and kidney in both sexes after the single low dose and in males after the repeated low dose and (2) in the liver, kidney and stomach in both sexes after the single high dose and in females after the repeated dose. Comparable amounts of radiolabel were found in the tissues following repeated and single low doses. NOTE: Whole blood and plasma values on a µg equivalents/g or equivalent/mL basis were not provided.

At the 6- and 24-hours sacrifice times, higher levels of radiolabel were observed in the tissues than at the 4-day sacrifice. Six hours after the single low dose, 4 to 7% of the administered dose was found in the tissues. Following the single high dose, from 2 to 3% of the administered dose was present in the tissues at 24 hours.

Table 5. Tissue Distribution [ppm; µg equivalents/g tissue]

Regimen / Sex / Tissue	LOW DOSE		HIGH DOSE		REPEATED DOSE	
	Males	Females	Males	Females	Males	Females
Heart	0.1394	0.1513	0.8640	0.9241	0.1572	0.1903
Lung	0.1149	0.1727	1.2378	1.6181	0.2400	1.5437
Spleen	0.2643	0.4623	1.7077	2.2045	0.6831	1.2175 [0.3142]♥
Kidney	1.3245(0.062)♦	1.6353(0.075)	4.260(0.017)	8.618(0.033)	0.6985(0.027)	0.8813(0.033)
Liver	1.0376(0.215)	1.6255(0.344)	7.792(0.147)	11.597(0.213)	1.1680(0.249)	1.7538(0.340)
Fat	0.1590	0.2913	1.1876	1.8528	0.2386	0.6255
Gonads	0.0434	0.3289	0.4312	2.5386	0.0889	0.6583
Muscle	0.0369	0.0463	0.3960	0.4031	0.0681	0.0879
Brain	0.0191	0.0285	0.3089	0.3360	0.0391	0.0671
Bone Marrow	0.2951	0.3788	2.295	2.224	0.3292	0.2881
Adrenal	0.6959	0.8847	6.0787	6.5789	0.6197	0.8539
Thyroid	0.2853	0.3223	3.4845	2.9579	0.3843	0.4066
Stomach	0.1081(0.003)	0.2752(0.007)	5.378(0.015)	14.686(0.117)	0.4517(0.010)	1.4936)0.036)
Intestine	0.2901(0.007)	0.3109(0.011)	2.508(0.006)	2.914(0.009)	0.4423(0.007)	0.5192(0.009)
TOTAL (%)♦♣*	**0.298**	**0.455**	**0.196**	**0.384**	**0.313**	**0.482**

Regimen / Sex / Tissue	LOW DOSE - 6 hr post-dose sacrifice		HIGH DOSE 24 hr post-dose sacrifice	
	Males	Females	Males	Females
Heart	0.4585	0.4860	2.4510	2.4427
Lung	3.1969	1.0997	5.4573	7.6268
Spleen	1.1836	1.0711	4.2915	5.6555
Kidney	4.6912(0.153)	4.6424(0.156)	18.817(0.059)	18.573(0.054)
Liver	12.519(2.075)	20.074(3.230)	44.902(0.663)	56.719(0.787)
Fat	0.2207	0.4621	7.7910	2.0224
Gonads	0.1409	2.2319	2.7088	9.7554
Muscle	0.2885	0.3228	2.2232	2.4170
Brain	0.0774	0.0795	0.7084	0.6354
Bone Marrow	1.0799	0.4162	5.7472	5.7870
Adrenal	1.5862	1.1276	9.3899	8.8891
Thyroid	1.3675	0.6979	10.576	7.6244
Stomach	58.230(1.362)	76.474(2.174)	572.157(1.819)	453.395(1.379)
Intestine	53.997(0.763)	47.713(0.962)	70.692(0.108)	46.552(0.098)
TOTAL (%)♦♣*	**4.432**	**6.578**	**2.677**	**2.348**

♦ (% of administered dose); ♣ includes whole blood values♥ [value with outlier omitted]; * 4 days post dose

Table 6. Half-life of Elimination of ^{14}C

Dose Group	Whole Blood $t_{1/2}$ (hours)	Plasma $t_{1/2}$ (hours
Low dose ♂	15.9	16.1
High dose ♂	25.0	19.4
Repeated ♂	?	?
Low dose ♀	26.6	20.5
High dose ♀	48.4	25.0
Repeated ♀	?	?

DISCUSSION

The elimination of ^{14}C-RH-287 Technical following three different dosing (oral) schedules [single high (250 mg/kg), single low (20 mg/kg), and single low (20 mg/kg) after 14-day repeated dosing with non-radiolabeled RH-287 Technical at 20 mg/kg] was similar among the groups, and there was no significant sex difference. The major route of excretion was via the feces, with the total percent of the dose excreted (urine and feces combined) being ≈89% for males/≈94% females [single low dose] and ≈95% for males/≈92% for females [single high dose] at 4 days post dose. Tissue levels were low, but detectable, in all groups and in all tissues measured (0.019-11.6 ppm) at 96 hours

SD

[4 days]. In general, the tissue levels were comparable following the single low- and repeat low-dose schedules. The high-dose animals displayed higher tissue values (on ppm basis) than the low-dose animals, but the ratio of radiolabel concentration in the tissues varied from 3.2-49.8 in ♂♂ and 4.8-53.4 in ♀♀. The ratio of the high to low dose is 12.5. With regard to the "elimination $T_{1/2}$" in whole blood at the high dose, the value for the females is nearly twice that for males, suggesting a sex difference. TB II disagrees with this determination, although the author also concludes that there are no sex differences. No information was provided on how the $T_{1/2}$ values were determined. Figure 8 displays the whole blood concentrations of radiolabel over time. The 24-hour mark for the females exceeds that of the males, while the other time points are generally comparable between the sexes. Considering the individual values [page 039 of the report], females 01646-45 and 01646-46 show an increase of considerable magnitude [14.8 and 11.8] between 10 hours and 24 hours compared to all other changes with time for all groups and time points, suggesting that these values may be spurious. It appears that a more appropriate $T_{1/2}$ would be derived using the last 3 data points for each sex, since at earlier time points absorption may still be occurring. The highest percentage of the radiolabel was observed in the liver and kidneys at 96 hours following all dosing schedules, although the high and repeat dose females also displayed slightly higher values in the stomach than in the kidneys.

Table 7. Ratio of High-:Low-Dose Tissue Levels

TISSUE/ORGAN	Males	Females
Heart	6.2	6.1
Lung	10.8	9.4
Spleen	6.5	4.8
Kidney	3.2	5.3
Liver	7.5	7.1
Fat	7.5	6.4
Gonads	9.9	7.7
Muscle	10.7	8.7
Brain	16.2	11.8
Bone Marrow	7.8	5.9
Adrenal	8.7	7.4
Thyroid	12.2	9.2
Stomach	49.8	53.4
Intestine	8.7	9.4

CONCLUSION

The majority (81-93%) of the administered dose of radiolabeled RH-287 Technical following single oral exposures to 20 mg/kg or 250 mg/kg was eliminated within 2 days, and the major route of elimination was via the feces. Similarly following a 14-day repeated 20 mg/kg/day dose, 60-70% of a single 20 mg/kg dose was eliminated within 2 days. Radiolabel was distributed to all tissues measured. The concentration of radiolabel remaining in the tissues at 96 hours was dose-related and represented ≈ 0.3% ♂♂/<0.5% ♀♀ of the administered dose. At 4 days post dose, the highest percentage of the dose was displayed in the liver, kidney, and stomach. The overall recovery of radiolabel following single-dose exposure was 90%-96%. Overall recovery following repeated dosing was lower [64%-74%], but this was attributed to differences in fecal homogenate preparation. No sex

differences were observed, and pretreatment for 14 days did not alter the elimination of the radiolabel. It does not appear that RH-287 Technical would bioaccumulate significantly.

This study is classified Acceptable. The identification and characterization of the metabolites is to be reported in a separate report. This study does not satisfy the guideline requirement (85-1) for a metabolism study, but with the submission of an acceptable metabolite identification report, this data requirement will be satisfied.

4,5-DICHLORO-2-N-OCTYL-3(2H)-ISOTHIAZOLONE SALMONELLA

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II/HED 7509C
EPA Section Head: James N. Rowe, Ph.D.
Review Section III,
Toxicology Branch II/HED 7509C

Signature: Nancy L. McCarroll
Date: 4-26-95
Signature: James N. Rowe
Date: 4/26/95

DATA EVALUATION REPORT

STUDY TYPE: Salmonella typhimurium mammalian/microsome mutagenicity assay

DP BARCODE: D213676

SUBMISSION NO.: S478172

PC CODE: 128101

MRID NUMBER: 434716-05

TEST MATERIAL: RH-287 technical

SYNONYM(S): 4,5-Dichloro-2-n-octyl-3(2H)-isothiazolone

STUDY NUMBER(S): Report No. 93R-0230

SPONSOR: Rohm and Haas Co. Spring House, PA

TESTING FACILITY: Rohm and Haas Co. Spring House, PA

TITLE OF REPORT: RH-287 Technical: Salmonella typhimurium Gene Mutation Assay (Ames Test)

AUTHOR(S): Sames, J. L. and Elia, M. C.

REPORT ISSUED: October 31, 1994

CONCLUSIONS--EXECUTIVE SUMMARY: In two independent microbial gene mutation assays (MRID No. 434716-05), Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 were exposed to nonactivated doses of 0.3, 1, 3, 10 or 30 μg/plate (initial trial) or 0.1, 0.3, 1, 3 or 7.5 μg/plate (confirmatory trial) or S9-activated doses of 3, 10, 30, 100 or 300 μg/plate (initial trial) or 1, 3, 7.5, 30 or 75 μg/plate (confirmatory trial). S9 activation was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in acetone.

Cytotoxicity was achieved at levels ≥ 3 μg/plate -S9 and ≥75 μg/plate +S9. **There was, however, no evidence of a mutagenic response at any dose either with or without S9 activation in either trial.** All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

CLASSIFICATION: Acceptable

The study is classified as **Acceptable** and satisfies the guideline requirement for a microbial gene mutation assay (84-2).

A. MATERIALS:

1. Test Material: RH-287 technical

Description: Not described but assumed to be a tan to brown solid (see MRID Nos. 434716-06 and 07)
Lot/ batch number: 4086 (TD No. 93-115)
Purity: 98.8% a.i.
Receipt date: Not listed
Stability: Stable in the solvent (acetone) when stored frozen for at least 8 months
CAS number: Not provided
Structure: Not provided
Solvent used: Acetone
Other comments: Test material storage conditions were not reported. Dosing solutions were adjusted to 100% a.i.; the frequency of solution preparation was not reported. Samples of the dosing solutions were stored frozen for stability and concentration analyses.

2. Control Materials:

Negative: None
Solvent/final concentration: Acetone--0.1 ml/plate
Positive: Nonactivation:
Sodium azide 2 µg/plate TA100, TA1535
2-Nitrofluorene 3 µg/plate TA98
9-Aminoacridine 100 µg/plate TA1537
Other:

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

3. Activation: S9 derived from unspecified strain, weight or sex

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

The rat liver S9 homogenate (Lot No. 0425) was obtained commercially from Molecular Toxicology Inc. The composition of the S9-cofactor mix was as follows:

Component	Concentration
Sodium phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
$MgCl_2$	8 mM
KCl	33 mM
S9	10%

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

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

5. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Six levels without S9 (0.03, 0.1, 0.3, 1, 3 or 10 μg/plate) and six levels with S9 (1, 3, 10, 30, 100 and 300 μg/plate) were evaluated in duplicate using strain TA100.

(b) Mutation assays:

Initial assay: Five nonactivated doses (0.3, 1, 3, 10 and 30 μg/plate) and five S9-activated doses (3, 10, 30, 100 and 300 μg/ml) were evaluated in all tester strains. Triplicate plates were prepared per test material dose per strain per condition. Six replicate plates per strain with or without S9 activation were prepared for the solvent and positive controls.

Confirmatory assay: 0.1, 0.3, 1, 3 and 7.5 μg/ml -S9 or 1, 3, 7.5, 30 or 75 μg/plate were processed as described for the initial assay.

B. TEST PERFORMANCE:

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

(a) Mutation Assays: To tubes containing 2 ml of molten top agar the following were added: 0.1 ml of a 10-hour broth culture (10^8-10^9 cells/ml) of the appropriate tester strain, 0.1 ml of the appropriate test material dose, solvent, or positive controls and 0.5 ml of phosphate buffer mix (nonactivated tests) or 0.5 ml of S9-cofactor mix (S9-activated tests). The contents of each tube were mixed, poured over minimal-glucose medium, and incubated at 37°C ± 1°C for ≈72 hours. At the end of incubation, the background lawn of growth

was examined and revertant colonies were counted. Means and standard deviations were calculated. A sterility test was performed on unspecified materials.

(b) Evaluation criteria:

(1) Assay validity: The assay was considered valid if the following criteria were met: (1) the presence of the appropriate genetic markers was verified for each strain; (2) the number of spontaneous revertants of each strain fell within the reporting laboratory's provided acceptable ranges; and (3) all positive controls induced a positive response.

(2) Positive response: The test material was considered positive if it caused a reproducible ≥2-fold increase in revertant colonies of any strain.

C. REPORTED RESULTS:

1. Analytical Determinations: The concentration of RH-287 in dosing solutions prepared for both trials of the mutation assay was generally within ±10% of the nominal values.

2. Preliminary Cytotoxicity Assay: Doses of 0.3 to 10 μg/plate -S9 and 1 to 300 μg/plate +S9 were assayed for cytotoxic effects on strain TA100. The highest nonactivated dose (10 μg/plate) and the two highest S9-activated doses (100 and 300 μg/plate) were cytotoxic. Based on these findings, the initial mutation assay was performed with half-log dilutions of the test material ranging from 0.3-30 μg/plate -S9 and 3-300 μg/plate +S9.

2. Mutation Assays: As shown in study report, Table I, nonactivated doses ≥10 μg/plate were severely cytotoxic in all strains, and reduced revertant colony counts were apparent for all strains at 3 μg/plate. Cytotoxicity was also severe at RH-287 levels ≥100 μg/plate +S9. There was, however, no appreciable increase in histidine revertants of any strain at noncytotoxic doses either in the presence or absence of S9 activation. Similar evidence of cytotoxicity for the majority of strains was observed at ≥3 μg/plate -S9 and 75 μg/plate +S9 in the confirmatory trial using a narrower range of concentrations (see attached study report, Table II). Also in agreement with the initial findings, the test material was not mutagenic. By contrast to the negative results with the test material, all strains responded in the expected manner to the appropriate nonactivated or S9-activated positive controls.

Based on the overall results, the study authors concluded that RH-287 was negative in this microbial test system.

D. REVIEWER'S DISCUSSION/CONCLUSIONS: We assess that the mutation assays were properly conducted and that the study authors interpreted the data correctly. RH-287 was tested to cytotoxic levels (≥3 μg/plate -S9; ≥75 μg/plate +S9) with no evidence of mutagenic effect in two independently performed trials. The

response of all strains to the appropriate nonactivated and S9-activated positive controls demonstrated the sensitivity of the test system to detect mutagenesis. We concluded, therefore, that the study provided acceptable evidence that the test material was negative in this microbial gene mutation assay.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated October 24, 1994).

F. APPENDIX ATTACHED: Tables I and II, Study Report pp. 12 and 13.

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Page _____ is not included in this copy.

Pages 58 through 59 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

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

_____ The document is not responsive to the request.

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.

4,5-DICHLORO-2-N-OCTYL-3(2H)-ISOTHIAZOLONE MICRONUCLEUS

EPA Reviewer: Nancy McCarroll Signature: Nancy McCarroll
Review Section III,
Toxicology Branch II/HED 7509C Date: 5/2/95
EPA Section Head: James N. Rowe, Ph.D. Signature: James N Rowe
Review Section III,
Toxicology Branch II/HED 7509C Date: 5/3/95

DATA EVALUATION REPORT

STUDY TYPE: In vivo micronucleus assay in mice

DP BARCODE: D213676

SUBMISSION NO.: S478172

PC CODE: 128101

MRID NUMBER: 434716-08

TEST MATERIAL: RH-287 technical

SYNONYM(S): 4,5-Dichloro-2-n-octyl-3(2H)-isothiazolone

STUDY NUMBER(S): Report No. 93P-232

SPONSOR: Rohm and Haas Co. Spring House, PA

TESTING FACILITY: Rohm and Haas Co. Spring House, PA

TITLE OF REPORT: RH-287 Technical: Micronucleus Assay in CD-1 Mouse Bone Marrow Cells

AUTHOR(S): Sames, J. L. and Elia, M. C.

REPORT ISSUED: September 12, 1994

CONCLUSIONS--EXECUTIVE SUMMARY: In an in vivo micronucleus assay (MRID No. 434716-08), groups of CD-1 male and female mice (5, 5 or 7/dose/sex/sacrifice time) received single oral gavage administrations of 32.5, 162.5 or 325 mg/kg a.i. of RH-287, respectively. Bone marrow cells were examined 24 and 48 hours posttreatment for the frequency of micronucleated polychromatic erythrocytes (MPEs). The test material was delivered to the test animals in corn oil.

The observation of mild and transient clinical signs (passive behavior and scant feces in high-dose males and females) suggests that the maximum tolerated dose (MTD) was not achieved. There was also no evidence of target cell cytotoxicity at any dose or harvest time. A slight but dose-related increase in MPEs was observed in the males of the mid- and high-dose groups at the 24-hour sacrifice. The increase was significant ($p<0.05$) at 325 mg/kg. However, the findings are only suspect and do not provide sufficient evidence to classify RH-287 as clastogenic/aneugenic in this test system. This issue can only be resolved by exposing the test animals to the MTD.

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CLASSIFICATION: Unacceptable

The study is classified as Unacceptable and does not satisfy the guideline requirement for a micronucleus assay (84-2).

A. MATERIALS:

1. Test Material: RH-287 technical

Description: Not described but assumed to be a tan to brown solid (see MRID Nos. 434716-06 and 07)
Lot/ batch number: 4086 (TD No. 93-115)
Purity: 98.8% a.i.
Receipt date: Not listed
Stability: Stable in the vehicle (corn oil) when stored at refrigerator temperatures for at least 11 days
CAS number: Not provided
Structure: Not provided
Vehicle used: Corn oil
Other comments: Test material storage conditions were not reported. Dosing solutions were adjusted to 100% a.i.; the frequency of solution preparation was not reported. Samples of the dosing solutions were stored refrigerated for stability and concentration analyses.

2. Control Materials:

Negative: None

Vehicle/final concentration/route of administration: Corn oil/ 10 ml/kg/oral gavage

Positive/final dose(s)/route of administration: Mitomycin C was dissolved in distilled water and administered at concentrations of 0.35 or 2.0 mg/kg by intraperitoneal injection.

3. Test Compound:

Route of administration: Oral gavage

Volume of test substance administered: 10 ml/kg

Dose levels used: 32.5, 162.5 and 325 mg/kg a.i. (The high dose represents the $\approx LD_{10}$)

Note: Dose selection was based on the findings of an acute oral toxicity study (Rohm and Haas Report No. 94R-0003; see Data Evaluation Report for MRID NO. 434716-01) in male and female CD-1 mice which indicated an approximate LD_{10} and LD_{50} for RH-287 of 329 and 567 mg/kg, respectively.

4. Test Animals:

(a) Species: Mouse Strain: CD-1 Age: ≈50 days
Weight range : 21-28 g
Sex: Males and females Source: Charles River Laboratories, Inc., Portage, MI .

(b) Number of animals used per dose:

- Treatment groups: 10 males 10 females
- Positive control: 5 males 5 females
- Vehicle control: 10 males 10 females

An additional group of four animals (2 males and 2 females) per unit time received the high dose and were used in the event of unscheduled deaths in the primary group animals. These additional animals were, however, included in the bone marrow cell evaluation.

(c) Were test animals properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and sampling times:

(a) Test compound:
Dosing: x once _____ twice (24 hours apart)
_____ other (describe):
Sampling (after last dose): _____ 6 hours _____ 12 hours
x 24 hours x 48 hours ____ 72 hours

(b) Vehicle control:
Dosing: x once _____ twice (24 hours apart)
_____ other (describe):
Sampling (after last dose): x 24 hours x 48 hours
____ 72 hours

(c) Positive control:
Dosing: x once _____ twice (24 hours apart)
_____ other (describe):
Sampling (after last dose): x 24 hours _____ 48 hours
_____ 72 hours

3. Tissues and Cells Examined:

x bone marrow _____ other (list):
Number of polychromatic erythrocytes (PCEs) examined per animal:
≈2000

Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: Number observed in at least 1000 total erythrocytes

4. Details of Cell Harvest and Slide Preparation: At 24 and 48 hours after the administration of the test material or vehicle control, the appropriate groups of animals were sacrificed by cervical dislocation. Animals in the positive control group were sacrificed 24 hours postexposure. The bone marrow from both femurs of each animal was flushed from the femurs, mixed with fetal bovine serum (FBS) and centrifuged. Cell pellets were mixed with residual FBS and spread onto slides. Slides were air-dried, fixed in methanol, stained with acridine orange and coded. The slides were scored for micronuclei in polychromatic erythrocytes (MPEs), and the ratio of PCEs to NCEs was determined.

5. Statistical Methods: The percentage of MPEs per group was transformed using an arcsine root transformation and these data were analyzed using a three-way analysis of variance and Dunnett's t-test. The above statistical procedures were used to analyze the untransformed PCE:NCE data. Data were analyzed separately for each sex; p-values <0.05 were considered significant.

6. Evaluation Criteria: The test material was considered positive if it induced a positive dose response and a statistically significant increase in the percentage of MPEs over the concurrent vehicle control values at one or more doses.

C. REPORTED RESULTS:

1. Analytical Determinations: The actual concentration of RH-287 in the three dosing solutions ranged from 103% of the target level for the high dose (325 mg/kg) to 115% for the low dose (32.5 mg/kg).

2. Micronucleus Assay:

(a) Animal observations: Animals were observed for clinical signs during the treatment period and prior to sacrifice. No unscheduled deaths occurred in any experimental group. Clinical signs of toxicity were reported to be consistent with those seen in the acute oral toxicity study and included: passive behavior and brown/yellow stains around the anogenital region in the majority of high-dose animals on Day 0. Scant feces was also reported for all high- and mid-dose males and females on Day 1. All clinical signs were transient in nature and resolved by Day 2.

(b) Bone marrow observations: PCE:NCE ratios were comparable among test and vehicle control groups at both harvest times (Table 1). A significant ($p<0.05$) increase in MPEs was observed in bone marrow cells harvested from male mice 24 hours after administration of the high dose (325 mg/kg). Initially, the study authors discounted this

increase as being biologically relevant because of the "relatively low number" of MPEs in the vehicle control males. No historical data were provided to support this claim. Subsequently, the study authors cited the work of Salamone and Mauvourin (1994)[1] on the mean spontaneous MPE frequencies of mouse stocks including CD-1 mice (0.1-0.3% MPEs) to justify the conclusion that RH-287 was negative in this test system. The use of published spontaneous MPEs is not recommended because background control data vary widely among laboratories. Nevertheless, our reviewers noted that the values for male mice in the vehicle control group (0.11% MPEs --24 hours; 0.13% MPEs--48 hours) were comparable to the mean frequency (0.13%) reported by Salamone and Mauvourin (1994)[2] for CD-1 mice. Therefore, using the concurrent background frequency for males (0.12% MPEs, combined for both harvest times) in conjunction with the criterion of Salamone et al (1980)[3], which assumes a comparable spontaneous frequency, a mean group incidence of 0.26% MPEs for seven animals would be considered suggestive of a positive response. As shown in Table 1, the significantly increased frequency of MPEs (0.28%) for high dose males at the 24-hour interval exceeded this value. Similarly, the MPE frequency at 24 hours for mid-dose males (0.23%) is suspect but lower than the presumptive positive value for a group containing five animals. There was, however, no evidence of a genotoxic response in females at any dose or harvest time or in males exposed to the low dose. The study authors concluded that RH-287 was not genotoxic in this in vivo mouse micronucleus assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We disagree with the study authors' assessment that RH-287 was negative in this in vivo assay. We have concerns related to the selection of the high dose as well as the significant increase (<0.05) in MPEs scored in high-dose males (24 hours). The study authors stated that the LD_{10} was chosen as the high dose because it was "expected to produce significant toxicity". We contend that the observed clinical signs (i.e., passive behavior and scant feces), while consistent with those seen in the acute oral study (MRID No. 43716-01) at ≥1000 mg/kg (both sexes) and at 500 mg/kg in females, were mild and transient in nature. In addition, deaths observed in the acute oral study at the above levels did not occur until 3 days posttreatment. The lack of "significant toxicity" in conjunction with the findings from the acute oral study indicated to us that the mice in the micronucleus assay could have tolerated a higher dose. It is also conceivable that the relevance,

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[1]Salamone, M.F. and Mavournin, K.H. (1994). Bone marrow micronucleus assay: A review of the mouse stocks used and their published mean spontaneous micronucleus frequencies. Environ. Mol. Mutagen. 23:239-273.

[2]Ibid.

[3]Salomone, M.F., Heddle, J.A., Katz, M. (1980). Mutagenic activity of 41 compounds in the in vivo micronucleus assay. In: F.J. de Serres and J. Ashby, eds. Progress in Mutation Research, Vol.1, Elsevier, North Holland, 686-697.

if any, of the significant increase in micronuclei induction noted in high dose-males could have been resolved by exposing the test animals to the MTD. Based on these considerations, we conclude that the study is unacceptable.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated September 12, 1994.)

F. APPENDIX Attached: No.

TABLE 1. Representative Results of the Micronucleus Assay in Mice with RH-287

Substance	Dose/kg	Exposure Time[a] (hours)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group	Number of MPEs per Group	Mean Percent MPEs ± S.D.	Mean PCE/NCE Ratio ± S.D.
Vehicle Control								
Corn oil	10 mL	24	M	5	10,560	12	0.11 ± 0.06	1.92 ± 0.70
		48	M	5	10,385	13	0.13 ± 0.08	3.00 ± 1.10
	10 mL	24	F	5	10,685	23	0.21 ± 0.11	2.13 ± 0.55
		48	F	5	10,380	18	0.17 ± 0.13	2.94 ± 0.94
Positive Control								
Mitomycin C[b]	0.35 mg	24	M	5	10,230	74	0.72 ± 0.32*	1.94 ± 0.36
		24	F	5	10,370	73	0.70 ± 0.19*	2.32 ± 1.00
Test Material								
RH-287	162.5 mg[c]	24	M	5	10,345	24	0.23 ± 0.11	1.68 ± 0.61
	325.0 mg	24	M	7	14,686	41	0.28 ± 0.13*	1.64 ± 0.54
	162.5 mg[c]	48	M	5	10,420	16	0.15 ± 0.13	1.96 ± 0.30
	325.0 mg	48	M	7	14,854	23	0.16 ± 0.13	1.79 ± 1.08
	162.5 mg[c]	24	F	5	10,605	20	0.19 ± 0.07	2.82 ± 1.03
	325.0 mg	24	F	7	14,749	22	0.15 ± 0.05	2.27 ± 0.64
	162.5 mg[c]	48	F	5	10,430	15	0.14 ± 0.07	2.43 ± 0.82
	325.0 mg	48	F	7	14,441	15	0.10 ± 0.07	2.23 ± 0.56

[a]Time after compound administration by oral gavage
[b]Mitomycin C was also assayed at 2.0 mg/kg and induced a significant ($p<0.05$) response; data from the lower dose were selected as representative.
[c]No treatment-related toxic, cytotoxic, or clastogenic effects were seen in the low-dose group (32.5 mg/kg).

*Significantly higher ($p<0.05$) than the corresponding vehicle control by Dunnett's t-test.

Abbreviations used: PCE = polychromatic erythrocyte; MPE = micronucleated polychromatic erythrocyte; NCE = normochromatic erythrocyte

Note: Data were extracted from the CBI, pp. 15-21, 23-25, and 32-35.

4,5-DICHLORO-2-N-OCTYL-3(2H)-ISOTHIAZOLONE IN VITRO CHROMOSOME ABERRATION

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II/HED 7509C
Signature: Nancy E.M. Carroll
Date: 5/2/95
EPA Section Head: James N. Rowe, Ph.D.
Review Section III,
Toxicology Branch II/HED 7509C
Signature: James N Rowe
Date: 5/3/95

DATA EVALUATION REPORT

STUDY TYPE: In vitro mammalian cytogenetics chromosome aberration in Chinese hamster ovary (CHO) cells

DP BARCODE: D213676

SUBMISSION NO.: S478172

PC CODE: 128101

MRID NUMBER: 434716-07

TEST MATERIAL: RH-287 technical

SYNONYM(S): 4,5-Dichloro-2-n-octyl-3(2H)-isothiazolone

STUDY NUMBER(S): SITEK No. 0258-3114; Sponsor's Report No. 93RC-233

SPONSOR: Rohm and Haas Co. Spring House, PA

TESTING FACILITY: SITEK Research Laboratories, Rockville, MD

TITLE OF REPORT: Test for Chemical Induction of Chromosome Aberration in Cultured Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation

AUTHOR(S): Kumaroo, P.V.

REPORT ISSUED: November 4, 1994

CONCLUSIONS--EXECUTIVE SUMMARY: In two independent Chinese hamster ovary in vitro cytogenetic assays (MRID No. 434716-07), cell cultures were exposed to nonactivated doses of 0.3, 0.6 or 0.7 μg/ml (initial trial) or 0.5, 0.6 or 0.7 μg/ml (confirmatory trial) or S9-activated concentrations of 6.0, 7.0 or 8.0 μg/ml (both trials). Cell harvest times were: 23 hours (initial nonactivated trial), 23 and 47 hours (confirmatory nonactivated trial), 20 hours (initial S9-activated trial), or 20 and 44 hours (confirmatory S9-activated trial). The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in acetone.

Cytotoxicity, as indicated by reduced mitotic indices (≈50%), was seen at ≥0.7 μg/ml -S9 and 8.0 μg/ml +S9; interference with cell-cycle kinetics was also observed at nonactivated doses as low as 0.05 μg/ml and at S9-activated

levels ≥4.0 μg/ml. Slight increases in the frequency of cells bearing structural aberrations, particularly complex aberrations, were noted in cultures harvested after the prolonged recovery from exposure to the highest nonactivated or S9-activated dose. Complex structural aberrations were also scored at the remaining doses +/-S9. However, the increases in aberrant cells never exceeded 3% of the examined population and never approached a level of statistical significance. **The evidence is, therefore, not sufficient to conclude that RH-287, tested up to cytotoxic levels, induced a clastogenic response in the presence or absence of S9 activation.**

CLASSIFICATION: Acceptable

A. MATERIALS:

1. Test Material: RH-287 technical

Description: Tan to brown waxy solid
Lot/ batch number: 4086 (TD No. 93-115)
Purity: 98.8% a.i
Receipt date: November 4, 1993
Stability: Stable in the solvent (acetone) when stored frozen for at least 8 months
CAS number: Not provided
Structure: Not provided
Solvent used: Acetone
Other comments: The test material was stored at room temperature. Dosing solutions were corrected for 100% a.i. and prepared immediately prior to use. Samples of the dosing solutions were shipped frozen to the sponsor for stability and concentration analysis.

2. Control Materials:

Negative: Modified McCoy's 5A medium supplemented with fetal bovine serum (FBS) (used only in the absence of S9), L-glutamine, and antibiotics.

Solvent/final concentration: Acetone at a final concentration of 0.5% was the solvent control.

Positive:

(a) Nonactivation (concentrations, solvent): Mitomycin C (MMC) was dissolved in deionized, distilled water (DH_2O) and used at 0.12 μg/ml.

(b) Activation (concentrations, solvent): Cyclophosphamide (CP) was dissolved in DH_2O and used at 10.0 μg/ml.

3. Activation: S9 derived from 4-6-week old male Sprague-Dawley

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

The S9 was prepared by the performing laboratory (SITEK Batch No. 051993), and was found to have a protein concentration of 35.6 mg/ml. Prior to use, the S9 was evaluated for its potential to activate CP to a form that induced chromosome aberrations in CHO cells.

S9 mix composition:

Component
4 mM NADP
5 mM glucose-6-phosphate
30 mM KCl
10 mM $MgCl_2$
50 mM Sodium phosphate buffer (pH 7.4)
S9 100 µl/ml

4. Test Compound Concentration Used:

(a) Preliminary cytotoxicity assays: Four range-finding preliminary cytotoxicity tests were conducted; doses used were as follows:

Trial 1: 0.25, 0.83, 2.5, 8.3, 25, 83, 250, 833 and 2500 µg/ml with and without S9 activation

Trial 2: 0.025, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 µg/ml -S9
0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10 µg/ml +S9

Trial 3: 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 µg/ml -S9
0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 µg/ml +S9

Trial 4: 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 10 µg/ml +S9

(b) Cytogenetic assay:

(1) Initial assay: Seven nonactivated (0.1-0.7 µg/ml) and six S9-activated (3.0-8.0 µg/ml) doses were tested. Cultures exposed to 0.3, 0.6 or 0.7 µg/ml -S9 or 6.0, 7.0 or 8.0 µg/ml +S9 were evaluated for chromosome damage. Cells were harvested at 23 hours (-S9) and 20 hours (+S9).

(2) Repeat assay: Concentrations and harvested times were as listed above for the initial assay. However, a second cell harvest (47 hrs. -S9; 44 hrs. +S9) was included.

5. Test Cells: Chinese hamster ovary (CHO-WBL) cells originated at Litton Bionetics and were obtained from the Environmental Health and Testing Laboratory (Lexington, Kentucky). The cells were grown in

McCoy's 5A medium supplemented with FBS and L-glutamine. McCoy's 5A medium was also used for testing in the absence of S9 activation, and serum-free medium was used in the presence of S9. Cells were grown for ≈24 hours prior to treatment.

Properly maintained? Yes.

Cell line or strain periodically checked for mycoplasma contamination? Yes.

Cell line or strain periodically checked for karyotype stability? Yes.

B. TEST PERFORMANCE:

1. Cell Treatments: Cells were exposed to the test compound or positive or solvent controls for:

__21__ hours (nonactivated); __2__ hours (activated)

2. Preliminary Cytotoxicity Assay: Duplicate cultures, seeded at densities ranging from 5-10x10^5 cells/flask, were exposed in the presence or absence of S9 activation to the selected test material doses, the negative control (culture medium), or the solvent control (acetone). In the nonactivated assay, cells were treated for 22 hours. In the S9-activated assay, cells were treated for 2 hours, washed, fed complete culture medium and incubated for an additional 22 hours. In both phases of testing, colcemid (0.1 μg/ml) was added 2 hours before the cultures were harvested. Harvested cells were counted, swollen in hypotonic KCl, fixed in methanol:acetic acid (3:1), dropped onto slides and stained with Giemsa, and coded. Mitotic indices (MIs) were determined by counting the number of mitotic cells in a population of 500 cells/flask. For average generation time (AGT) determinations, cells were seeded at 4-8x10^5 cells/flask and treated as described above for the nonactivated conditions in the presence of 0.01 mM 5-bromo-2-deoxyuridine (BrdU). In the S9-activated phase of testing, cultures were treated as previously described and refed complete medium containing BrdU. Cells were harvested, swollen and fixed as described and stained by the modified fluorescence plus Giemsa method of Perry and Wolff.[1] One hundred metaphases from each dose level were analyzed for the number of cells in first division (M_1), between first and second division (M_{1+}), second division (M_2) and beyond second division (M_{2+}). Based on these findings, doses and harvest times were selected for the cytogenetic assay.

3. Cytogenetic Assay:

(a) Treatment: Duplicate cultures, seeded at 5x10^5 cells/flask were

[1]Perry, P. and S. Wolff. 1974. New Giemsa method for differential staining of sister chromatids. Nature 250:156-158.

exposed either in the presence or absence of S9 activation to the selected test material doses, the negative control, the solvent control (acetone) or the positive controls (MMC -S9; CP +S9). In the nonactivated assay, cells were dosed for 21 hours. In the S9-activated assay, cells were treated for 2 hours, washed, fed complete culture medium, reincubated, and harvested at 20 hours. In both phases of testing, colcemid (0.1 μg/ml) was added 2 hours before the cultures were harvested.

Metaphase cells were collected by trypsinization, fixed and stained as described for the preliminary cytotoxicity assay. Slides were coded prior to analysis.

For the confirmatory assay, four replicated cultures were exposed to the selected doses of the test material, negative, solvent or positive controls +/-S9 as described for the initial assay. Duplicate cultures per group were harvested at 23 or 47 hours (nonactivated conditions) or at 20 or 44 hours (S9-activated conditions).

(b) Metaphase analysis: Two hundred metaphase cells per treatment group (100 cells/culture) with 20-22 chromosomes were analyzed for chromosome aberrations. The number of polyploid or endoreduplicated cells in 100 dividing cells was also scored; structural aberrations were not analyzed in these cells. MIs were determined as described earlier. Gaps were counted but not included in the statistical analysis.

(c) Statistical analysis: The proportion of cells with aberrations was analyzed using the Chi-square test. Historical control data were used when the solvent control value was <1%. The Cochran-Armitage test was used to determine if there was a dose-related response. Significance was evaluated at $p \leq 0.05$.

(d) Evaluation criteria:

(1) Assay validity: The assay was considered valid if ≤4% of the solvent control cells were aberrant and ≥25% of the cells scored from the positive control cultures contained at least one chromosome aberration.

(2) Positive result: The test material was considered positive if it induced a reproducible, statistically significant ($p \leq 0.05$) increase in the percentage of cells with aberrations and the response was dose-related.

C. REPORTED RESULTS:

1. Solubility and Osmolality Tests: At the suggestion of the sponsor, the test material was melted at 40°C, weighed in liquid form and dissolved in acetone. RH-287 (476.2 mg) was soluble in 0.95 ml of

acetone, yielding a primary solution containing ≈ 500 mg/ml. Based on this result, the first range-finding assay was conducted with concentrations of 0.25 to 2500 μg/ml +/-S9. The osmolality of the treatment medium was also measured in the first range-finding assay and was found to be within acceptable ranges for all test material treatment levels.

2. Analytical Determination: All dosing solutions used in both cytogenetics assay were verified analytically. The actual concentration of the test material in the solutions prepared for the initial cytogenetic assay ranged from 99-152% of target. For the confirmatory trial, actual concentrations were 101-179% of the nominal values. The study author of the analytical report stated that the unexpected high RH-287 levels in several of the dosing solutions resulted from evaporation which occurred over the 6 months that the samples were stored prior to analysis.

2. Preliminary Cytotoxicity Assays: The study author stated that due to the "corrosive nature" of the test material, glass tissue culture vessels were used in placed of plastic flasks for all subsequent work. Based on the results of the solubility test, Trial 1 of the cytotoxicity assays was conducted with nine nonactivated and S9-activated doses ranging from 0.025-2500 μg/ml. Compound precipitation was observed at levels ≥250 μg/ml +/- S9. Owing to severe cytotoxicity, the assay was repeated. Trial 2 was not completed due to inclement weather; however, visual observation of the monolayers indicated severe cytotoxicity at doses ≥0.6 μg/ml -S9 and doses ≥6.0 μg/ml +S9. Accordingly, a third trial was conducted with nonactivated 0.05-0.5 μg/ml and S9-activated 0.5-5.0 μg/ml. Data from Trial 3 indicated that relative suspension growth (RSG) was reduced to 59 and 43% of the acetone control cultures at the two highest nonactivated levels (0.4 and 0.5 μg/ml, respectively). MIs were also reduced at these doses (67 and 58% of control at 0.4 and 0.5 μg/ml, respectively. Lower nonactivated doses (≤0.3 μg/ml) had no clear cytotoxic effects. In the presence of S9 activation, cells were only slightly affected at the high dose, with decreases of 22 or 19% in either the RSG or MI, respectively. Since the cytotoxic response induced in the S9-activated phase of Trial 3 was not considered to be adequate, Trial 4, which included only an S9-activated phase, was conducted. Results from this trial with doses of 3.0-10 μg/ml +S9 showed that no cells survived treatment with the high concentration. For the remaining levels, decreases in RSG were dose related and ranged from 95% at the low dose to 21% at 8.0 μg/ml. A parallel dose-dependent effect on the MIs was also noted. Effects on cell cycle kinetics were also assessed in conjunction with each trial. Mitotic delay was apparent at all nonactivated doses in Trial 3, and the average generation time (AGT) at the highest nonactivated dose (0.5 μg/ml) was increased to 21.4 hours. In the presence of S9 activation, the AGT was also increased (19.4 or 20.1 hours) at 7.0 or 8.0 μg/ml, respectively. From the overall results, doses and harvest times selected for the initial cytogenetic assay were: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 μg/ml with a 23-hour cell harvest (nonactivated assay); 3.0, 4.0, 5.0, 6.0,

7.0 and 8.0 μg/ml with a 20-hour cell harvest (S9-activated assay).

3. Cytogenetic Assay:

(a) Initial assay: MIs were reduced ≈50% at the highest nonactivated (0.7 μg/ml) and S9-activated (8.0 μg/ml) doses. CHO cells exposed to 0.3, 0.6 or 0.7 μg/ml -S9 or 6.0, 7.0 or 8.0 μg/ml +S9 were selected for evaluation of chromosome aberrations. As the representative data presented in Table 1 indicate, the frequency of cells with structural aberrations was not significantly increased at any dose with or without S9 activation. The marginal increase in the percentage of aberrant cells and the presence of a cell bearing a single quadriradial at the highest S9-activated dose were not considered by our reviewers to provide sufficient evidence of a positive response.

(b) Confirmatory: Comparable doses and harvest times were evaluated in the confirmatory assay. In addition, parallel sets of cultures were harvested at 47 hours (nonactivated) or 44 hours (S9-activated). MIs, at the earlier harvest of cells treated with the highest nonactivated or S9-activated dose, were ≈50% lower than the MIs for the acetone controls (Table 2). Although recovery of posttreatment mitosis was improved after the second harvest, MIs were still appreciable lower than control (≈30%) at the high doses with or without S9 activation. As further shown in Table 2, no significant increases in the percentage of aberrant cells were seen at any nonactivated or S9-activated dose or harvest time. As noted for the initial assay, complex aberrations (2 quadriradials and 1 dicentric) were also scored at the highest S9-activated dose (20-hour harvest). The relevance of the quadriradials is somewhat diminished because a single quadriradial was also found in the solvent control cultures harvested at this time. It was further noted, however, that complex aberrations (e.g., triradials, quadriradials, dicentrics, rings, complex interchanges) were scored at all test material doses with or without S9 activation in cells harvested after the prolonged recovery time. In addition, a severely damaged cell (cell bearing ≥10 aberrations) was observed in cultures treated with 0.6 μg/ml -S9. Our reviewers also noted a slightly increased frequency of endoreduplicated mitosis at the two highest S9-activated levels (20-hour harvest) and a slight increase in polyploid cell at these RH-287 doses (+S9) after the prolonged recovery time. The study author did not comment on these unusual findings and concluded that RH-287 was negative in this test system.

The positive controls (MMC -S9 and CP +S9) induced significant ($p<0.001$) clastogenic responses in both assays.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that while a wide variety of rare complex aberrations were found in cultures harvested 47 or 44 hours after treatment with both the nonactivated and S9-activated test material,

the relevance of this finding must be interpreted with caution. The use of extremely narrow dose ranges in the confirmatory trial (0.5-0.7 μg/ml -S9; 5.0-8.0 μg/ml +S9) further confounds the interpretation of the results. Since the differences in the applied concentrations were minimal, it is not unreasonable to assume that essentially comparable levels of RH-287 were tested at each experimental point. Hence, there can be no doubt that the rare complex aberrations scored at all nonactivated and S9-activated doses in the second cell harvest were treatment related. The issue of whether these cytogenetic changes resulted from the test material directly attacking DNA or were a consequence of cytotoxicity must, therefore, be resolved. We tend to favor the latter explanation for the following reasons: (1) RH-287 was clearly shown to be severely cytotoxic and cause severe cell cycle delay, (2) even after the prolonged recovery time, MIs were still reduced by ≈30% which suggests that the aberrations may have been induced during the process of cell death and (3) increases in aberrant cells were marginal in all assays (never exceeding 3% and never approaching a level of statistical significance). Although greater confidence in the negative findings would have resulted from evaluating lower doses of RH-287, the slight increases in cells bearing chromosome aberrations, particularly complex aberrations, was not sufficient to conclude that the test material is clastogenic.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated November 4, 1994.)

F. APPENDIX ATTACHED? No

TABLE 1. Representative Results of the Initial Chinese Hamster Ovary (CHO) Cell *In Vitro* Cytogenetic Assay with RH-287

Substance	Dose/mL	S9 Activation	Harvest Time (Hours)	Mitotic Index[a]	No. of Cells Scored	Total Number of Aberrations[b]	Cells with Aberrations[b] (%)	Aberrations per Cell[b]	Biologically Significant Aberrations (No./Type)[c]
Negative Control									
Untreated cells	--	-	23	100	200	0	0.00	0.000	--
		+	20	100	200	3	1.00	0.015	2SB; 1D
Solvent Control									
Acetone	0.5%	-	23	100	200	1	0.50	0.005	1D
		+	20	100	200	0	0.00	0.000	--
Positive Control									
Mitomycin C	0.12 µg	-	23	53	200	140	46.0*	0.700	39TB; 2TBI; 22TR; 22QR; 12CR; 2ID; 26CI; 12SB; 2D; 1DM
Cyclophosphamide	10.0 µg	+	20	39	200	318	74.5*	1.590	46TB; 2TBI; 51TR; 57QR; 23CR; 5ID; 53CI; 39SB; 3D; 5R; 4DM; 3SD
Test Material									
RH-287	0.7 µg[d]	-	23	49	200	2	1.0	0.010	1TB; 1D
	8.0 µg[d]	+	20	52	200	8	2.5	0.040	5TB; 1QR;1SB; 1D

[a]Percent of corresponding solvent control
[b]Excluding gaps, polyploid cells, and endoreduplications

[c]Abbreviations used:
TB = Chromatid break
TBI= Isochromatid break
TR = Triradial
QR = Quadriradial
TE = Chromatid exchange
SB = Chromosome break
CR = Complex interchange
ID = Interstitial deletion
CI = Chromatid intrachange
SD = Severely damaged cell (i.e., ≥10 aberrations); counted as 10 aberrations
D = Dicentric
DM = Double minute
R = Ring

[d]There was no evidence of a clastogenic effect at lower doses (0.3 or 0.6 µg/ml -S9 or 6.0 or 7.0 µg/ml +S9).

*Significantly higher than the solvent control ($p<0.001$) by Chi-square test.

Note: Data were extracted from the study report pp. 49-51.

TABLE 2. Representative Results of the Confirmatory Chinese Hamster Ovary (CHO) Cell *In Vitro* Cytogenetic Assay with RH-287

Substance	Dose/mL	S9 Activation	Harvest Time (Hours)	Mitotic Index[a]	No. of Cells Scored	Total Number of Aberrations[b]	Cells with Aberrations[b] (%)	Aberrations per Cell[b]	Biologically Significant Aberrations (No./Type)
Negative Control									
Untreated cells	--	-	23	100	200	0	0.00	0.000	--
		-	47	100	200	1	0.50	0.005	1TB
	--	+	20	100	200	0	0.00	0.000	--
		+	44	100	200	0	0.00	0.000	--
Solvent Control									
Acetone	0.5%	-	23	100	200	1	0.50	0.005	1SB
		-	47	100	200	1	0.50	0.005	1D
		+	20	100	200	2	1.00	0.010	1QR; 1R
		+	44	100	200	3	1.50	0.015	1TB; 1SB; 1D
Positive Control[c]									
Mitomycin C	0.12 µg	-	23	48	200	141	46.5*	0.705	28TB; 4TBI; 28TR; 22QR; 3CR; 30CI; 10SB; 1D; 5DM; 1SD
Cyclophosphamide	10.0 µg	+	20	34	200	282	63.0*	1.410	41TB; 11TBI; 59TR; 33QR; 20CR; 7ID; 39CI; 38SB; 2D; 1R; 1DM; 3SD
Test Material									
RH-287	0.7 µg[d]	-	23	53	200	1	0.05	0.005	1TB
	0.5 µg	-	47	78	200	3	1.50	0.015	1TR; 1SB; 1DM
	0.6 µg	-	47	71	200	16	3.00	0.080	1TB; 1TR; 1CR; 2SB; 1D; 1SD
	0.7 µg	-	47	70	200	8	3.00	0.040	1TB; 2TR; 3CI; 1D; 1R
	8.0 µg[d]	+	20	47	200	6	2.50	0.030	2QR; 3SB; 1D
	6.0 µg	+	44	88	200	3	1.50	0.015	1TB; 1TR; 1D
	7.0 µg	+	44	80	200	4	1.50	0.020	2SB; 1D; 1R
	8.0 µg	+	44	73	200	9	3.00	0.045	1TB; 1TBI; 1TR; 1QR; 1CR; 1CI; 2SB; 1D

[a]Percent of solvent control
[b]Excluding gaps, polyploid cells, and endoreduplications
[c]Both positive controls induced powerful clastogenic effects at the later harvest; data from the first harvest were selected as representative.

4,5-DICHLORO-2-N-OCTYL-3(2H)-ISOTHIAZOLONE IN VITRO CHROMOSOME ABERRATION

[d]There was no evidence of a clastogenic effect at lower doses (0.5 or 0.6 µg/ml -S9 or 6.0 or 7.0 µg/ml +S9).

Abbreviations used:

TB = Chromatid break	SB = Chromosome break	SD = Severely damaged cell (i.e., ≥10 aberrations); counted as 10 aberrations
TBI= Isochromatid break	CR = Complex interchange	D = Dicentric
TR = Triradial	ID = Interstitial deletion	DM = Double minute
QR = Quadriradial	CI = Chromatid intrachange	R = Ring
TE = Chromatid exchange		

*Significantly higher than the solvent control ($p<0.001$)

Note: Data were extracted from the study report pp. 52-57.

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4,5-DICHLORO-2-N-OCTYL-3(2H)-ISOTHIAZOLONE

MAMMALIAN CELLS IN CULTURE
GENE MUTATION

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DATA EVALUATION REPORT

STUDY TYPE: Gene mutation in cultured mammalian cells (Chinese hamster ovary CHO cells)

DP BARCODE: D213676

SUBMISSION NO.: S478172

PC CODE: 128101

MRID NUMBER: 434716-06

TEST MATERIAL: RH-287 technical

SYNONYM(S): 4,5-Dichloro-2-n-octyl-3(2H)-isothiazolone

STUDY NUMBER(S): SITEK No. 0258-2500; Sponsor's Report No. 93RC-231

SPONSOR: Rohm and Haas Co. Spring House, PA

TESTING FACILITY: SITEK Research Laboratories, Rockville, MD

TITLE OF REPORT: Test for Chemical Induction of Gene Mutation at the HGPRT Locus in Cultured Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation

AUTHOR(S): Pant, K. J.

REPORT ISSUED: November 4, 1994

CONCLUSIONS--EXECUTIVE SUMMARY: In two independent Chinese hamster ovary (CHO) *in vitro* gene mutation assays (MRID No. 434716-06), cell cultures were exposed to nonactivated concentrations of 0.005, 0.025, 0.05, 0.1 or 0.5 μg/ml (initial trial) or 0.025, 0.05, 0.1, 0.2, 0.4, 0.5 or 0.75 μg/ml(confirmatory trial) or S9-activated doses of 0.5, 1.0, 2.5, 5.0, 10 or 25 μg/ml (initial trial) or 2.5, 5.0, 6.0, 8.0, 9.0, 10 or 15 μg/ml (confirmatory trial). The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in acetone.

In both trials, cytotoxicity was achieved at levels ≥ 0.5 μg/ml -S9 and ≥10 μg/ml +S9. There was, however, no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. CHO cells

responded in the expected manner to the nonactivated and S9-activated positive controls.

CLASSIFICATION: Acceptable

The study is classified as **Acceptable** and satisfies the guideline requirement for an in vitro mammalian cell gene mutation assay (84-2).

A. MATERIALS:

1. Test Material: RH-287 technical

Description: Tan to brown solid
Lot/ batch number: 4086 (TD No. 93-115)
Purity: 98.8% a.i.
Receipt date: November 4, 1993
Stability: Stable in the solvent (acetone) when stored frozen for at least 8 months
CAS number: Not provided
Structure: Not provided
Solvent used: Acetone
Other comments: The test material was stored at room temperature. Dosing solutions were adjusted to 100% a.i. and were prepared immediately prior to use. Samples of the dosing solutions were shipped frozen to the sponsor for stability and concentration analysis.

2. Control Materials:

Negative: None

Solvent/final concentration: Acetone -- 50 µl/ml

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in dimethyl sulfoxide to yield a final concentration of 0.5 µl/ml.

Activation (concentrations, solvent): 7,12-Dimethylbenz(a)anthracene (DMBA) was prepared in acetone to yield a final concentration of 5 µg/ml.

3. Activation: S9 derived from adult male Sprague-Dawley

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

Two batches of S9 homogenate (Batch Nos. 051993 and 020592), prepared by the performing laboratory, were used in the study. The protein content of each batch was 35.6 or 31.9 mg/ml protein, respectively.

Prior to use each batch was characterized for its ability to metabolize DMBA to a mutagen for CHO cells.

S9 mix composition:

Component	Concentration
NADP (sodium salt)	4 mM
Glucose-6-phosphate	5 mM
Potassium chloride	30 mM
Magnesium chloride	10 mM
Calcium chloride	10 mM
Sodium phosphate buffer, pH 7.5	50 mM
S9 homogenate	100 µl/ml of cofactor mix

4. Test Cells: Mammalian cells in culture

_____ mouse lymphoma L5178Y cells
__x__ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Yes.
Periodically checked for karyotype stability? Yes.
Periodically "cleansed" against high spontaneous background? Yes.

Cells were grown in Ham's F-12 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and antibiotics for 18-24 hours prior to use. Cell treatment was carried out in serum-free Ham'2 F-12.

5. Locus Examined:

_____ thymidine kinase (TK)
selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)

__x__ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
Selection agent: _____ 8-azaguanine (8-AG)
(give concentration) __10 µM__ 6-thioguanine (6-TG)

_____ Na^+/K^+ATPase
Selection agent: _____ ouabain
(give concentration)

_____ other (locus and/or selection agent; give details):

4. Test Compound Concentration Used:

(a) Preliminary cytotoxicity assays: Two range-finding preliminary cytotoxicity tests were conducted; doses used were as follows:

Trial 1: 0.1, 0.5, 1.0, 5.0, 10, 50, 100, 500, 1000 and 5000 μg/ml with and without S9 activation

Trial 2: 0.0025, 0.005, 0.01, 0.025, 0.05, 0.1 and 0.5 μg/ml-S9

(b) Mutation assays:

(1) Initial assay: Five nonactivated doses (0.005, 0.025, 0.05, 0.1 and 0.5 μg/ml) and six S9-activated doses (0.5, 1.0, 2.5, 5.0, 10 and 25 μg/ml) were tested. Cultures exposed to all levels except 25 μg/ml +S9 were cloned.

(2) Repeat assay: Concentrations tested were 0.025, 0.05, 0.1, 0.2, 0.4, 0.5 and 0.75 μg/ml -S9 and 2.5, 5.0, 6.0, 8.0, 9.0, 10 and 15 μg/ml +S9. Cultures exposed to all levels except 0.75 μg/ml -S9 or 15 μg/ml +S9 were cloned.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells exposed to test compound, solvent or positive controls for: 5 hours (nonactivated) 5 hours (activated)

(b) After washing, cells cultured for 8 to 9 days (expression period) before cell selection.

(e) After expression, $2x10^5$ cells/plate (5 plates) were cultured for 7 days in selection medium to determine numbers of mutants and 200 cells/plate (3 plates) for 7 days without selection medium to determine cloning efficiency.

2. Statistical Methods: Data were transformed and evaluated for statistical significance ($p \leq 0.05$) by ANOVA and Student's t-tests.

3. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if the following criteria were met: (1) the cloning efficiency (CE) of the solvent control was ≥65%; (2) the mutation frequency (MF) in the solvent control groups did not exceed 25 mutants/10^6 cells; (3) the MFs for the positive controls should exceed 100 mutants/10^6 cells.

b. Positive response: The test material was considered positive if it induced a significant and dose-related increase in the MF that was ≥2-fold higher than the concurrent and historical solvent controls.

C. REPORTED RESULTS:

1. Solubility, pH and Osmolality Determinations : At the suggestion of the sponsor, the test material was melted at 40°C, weighed in liquid form and dissolved in acetone. The test material (476.2 mg) was soluble in 0.95 ml of acetone, yielding a primary solution containing ≈ 500 mg/ml. Based on this result, the first range-finding assay was conducted with ten concentrations (0.1 to 5000 μg/ml +/-S9). RH-287 had no effect on the pH of the treatment medium. Similarly, the osmolality of the treatment medium containing 25 μg/ml of the test material (highest S9-activated dose in the initial trial) was measured and found to be within an acceptable range.

2. Analytical Determination: All dosing solutions used in both mutation assays were verified analytically. The actual concentration of the test material in the solutions prepared for the initial mutation assay ranged from 100-272% of target. For the confirmatory trial, actual concentrations were 99-114% of the nominal values. The study author of the analytical report stated that the unexpected high RH-287 levels in several of the dosing solutions resulted from evaporation which occurred over the 6 months that the samples were stored prior to analysis.

2. Preliminary Cytotoxicity Assays: Based on the results of the solubility test, Trial 1 of the cytotoxicity assay was conducted with ten nonactivated and S9-activated doses ranging from 0.1-5000 μg/ml. Compound precipitation was observed at levels ≥500 μg/ml +/- S9. In the absence of S9 activation, no cells survived treatment with test material levels ≥1.0 μg/ml. Relative cloning efficiency (RCE) for the remaining nonactivated doses (0.1 or 0.5 μg/ml) was ≤14%. Accordingly, the nonactivated phase of testing was repeated with doses of 0.0025-0.5 μg/ml. Survival in the repeat trial was generally dose dependent and ranged from 95% at 0.005 μg/ml to 23% at 0.5 μg/ml. In the presence of S9 activation, no cells were recovered from cultures exposed to ≥50 μg/ml. RCE at 10 μg/ml was 46%; lower levels (≤5.0 μg/ml) were not cytotoxic. From the overall findings, doses selected for the initial mutation assay were: 0.005-0.5 μg/ml -S9 and 0.5-25 μg/ml +S9. The study author stated that due to the "corrosive nature" of the test material, glass tissue culture vessels were used in placed of plastic flasks for all of the mutation assays.

3. Mutation Assays:

(a) Nonactivated conditions: Representative results from the nonactivated trials are presented in Table 1. The data from the cytotoxicity assessment performed concurrent with both nonacti-

vated trials were in good agreement and indicated that RCE posttreatment was reduced to ≤38% of the solvent control at 0.5 μg/ml. At the higher level used in the confirmatory assay (0.75 μg/ml), 11% of the cells were recovered; however, cells continued to die posttreatment, and these cultures were carried no further. As also shown in Table 1, no appreciable increase in the MF was observed at any dose in either the initial or confirmatory tests.

(b) S9-activated conditions: Representative data from the S9-activated assays (Table 2) confirm the results of the nonactivated trials and indicate that RH-287 was tested to cytotoxic levels (≥10 μg/ml) but failed to induce a mutagenic response in CHO cells.

By contrast to the negative results with RH-287, both the nonactivated (0.5 μl/ml EMS) and S9-activated (5.0 μg/ml DMBA) induced significant ($p \leq 0.5$) increases in the MF in both trials. Based on the results, the study author concluded that RH-287 did not induce gene mutations at the HGPRT locus in CHO cells.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that the study was properly conducted and that the study author correctly interpreted the data. RH-287 was evaluated to cytotoxic concentrations (0.5 μg/ml -S9; 10 μg/ml +S9) with no evidence of mutagenic activity in a well-conducted series of assays. The ability of the test system to detect a mutagenic response was also clearly demonstrated by the results obtained with the nonactivated and S9-activated positive controls. We conclude, therefore, that the study provided acceptable evidence that RH-287 was not mutagenic in this in vitro mammalian cell gene mutation assay.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A quality assurance statement was signed and dated November 4, 1994).

F. APPENDIX ATTACHED: NO.

TABLE 1. Representative Results of the Nonactivated CHO Forward Gene Mutation Assays with RH-287

Substance	Dose/mL	Relative % Cloning Efficiency (after treatment)	Total Mutant Colonies/ 5 Dishes	Cloning Efficiency (at selection)	Mutation Frequency/ 10^6 cells[a]
Solvent Control					
Acetone	50 µl[b]	100	7	0.78	9.0
	50 µl[c]	100	6	0.90	6.7
Positive Control					
Ethylmethane sulfonate	0.5 µl[b]	58	165[d]	0.55	333.3*
	0.5 µl[c]	14	263	0.48	547.9*
Test Material					
RH-287	0.5 µg[b,e]	38	4	0.82	4.9
	0.5 µg[c,e]	25	0[d]	0.79	0.0
	0.75 µg	11	--	--	--

[a]Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{\text{Number of Selection Plates (5) x Cloning Efficiency x 2 x } 10^5 \text{ cells}} \times 10^6$.

[b]Results for the initial assay.
[c]Results for the confirmatory assay.
[d]One of ten selection plates from the two replicate cultures was contaminated; calculations are based on mutants/4.5 selection plates.
[e]Findings for lower doses (0.005, 0.025, 0.05 or 0.1 µg/ml--initial assay or 0.025, 0.05, 0.1, 0.2, or 0.4 µg/ml-- confirmatory assay) did not suggest a mutagenic effect.

*Significantly higher ($p \leq 0.05$) than the solvent control by Student's t-test.
Note: Data were extracted from the study report, pp 32, 33, 35, 36, 44 and 48.

TABLE 2. Representative Results of the S9-activated CHO Forward Gene Mutation Assays with RH-287

Substance	Dose/mL	Relative % Cloning Efficiency (after treatment)	Total Mutant Colonies/ 5 Dishes	Cloning Efficiency (at selection)	Mutation Frequency/ 10^6 cells[a]
Solvent Control					
Acetone	50 µl[b]	100	3[c]	0.81	3.7
	50 µl[d]	100	7	0.92	7.6
Positive Control					
7,12 Dimethylbenz(a) anthracene	5.0 µg[b]	53	256	0.57	449.1*
	5.0 µg[d]	26	179[e]	0.48	414.4*
Test Material					
RH-287	10.0 µg[b,f]	38	3	0.76	3.9
	25.0 µg	0	--	--	--
	10.0 µg[d,f]	33	10[e]	0.89	12.5
	15.0 µg	2	--	--	--

[a]Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{\text{Number of Selection Plates (5) x Cloning Efficiency x 2 x } 10^5 \text{ cells}} \times 10^6$.

[b]Results for the initial assay.
[c]One of the replicate cultures was lost due to contamination.
[d]Results for the confirmatory assay.
[e]One of ten selection plates from the two replicate cultures was contaminated; calculations are based on mutants/4.5 selection plates.
[f]Findings for lower doses (0.5, 1.0, 2.5 or 5.0 µg/ml--initial assay or 2.5, 5.0, 6.0, 8.0 or 9.0 µg/ml--confirmatory assay) did not suggest a mutagenic effect.

*Significantly higher ($p \leq 0.05$) than the solvent control by Student's t-test.
Note: Data were extracted from the study report, pp 32, 34, 35, 37, 45 and 49.

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

April 17, 1995

SUBJECT: RH-287, Dermal Absorption in Rats

TO: Linda Taylor Ph.D.
Review Section II
Toxicology Branch II
Health Effects Division (7509C)

FROM: Robert P. Zendzian Ph.D.
Senior Pharmacologist
Toxicology Branch I
Health Effects Division (7509C)

Compound; RH-286 PC Code; 128101 MRID 434716-10

Submission; S478172 Registrant; Rohm and Haas DP Barcode; D212609

Action Requested

Review the following study;

Citation

^{14}C-RH-287 Dermal absorption study in male rats, V.S. Watts & G.A. Hazelton, Rohm and Haas, Toxicology Dept, Spring House, PA. Protocol No 92P-074, Report No. 92R-074. Feb 23, 1994. MRID 434716-10

Core Classification Unacceptable

Conclusions

The study is unacceptable. Test material was administered as a solution in acetone. The solvent used in applying a chemical to the skin has been found to produce significant qualitative and quantitative effects on the absorption process. For this reason the guideline for dermal absorption studies requires that the test chemical be dissolved or suspended in the vehicle to be used in the field. If no field vehicle has been determined, it is required that the test substance be dissolved or suspended in water.

Attachment DER

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Data Evaluation Report

Compound RH-287

Study type Dermal Absorption, FIFRA guideline 85-3

Citation

^{14}C-RH-287 Dermal absorption study in male rats, V.S. Watts & G.A. Hazelton, Rohm and Haas, Toxicology Dept, Spring House, PA. Protocol No 92P-074, Report No. 92R-074. Feb 23, 1994. MRID 434716-10

Reviewed by Robert P. Zendzian PhD
Senior Pharmacologist

Core Classification Unacceptable

Conclusions

The study is unacceptable. Test material was administered as a solution in acetone. The solvent used in applying a chemical to the skin has been found to produce significant qualitative and quantitative effects on the absorption process. For this reason the guideline for dermal absorption studies requires that the test chemical be dissolved or suspended in the vehicle to be used in the field. If no field vehicle has been determined, it is required that the test substance be dissolved or suspended in water.