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

009636

JUL 2 9 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Kathon® 287T Industrial Mildewcide (707-EEU)
Submission of 28-Day Oral/2-Week Recovery Study
in Support of the Registration

TO: John Lee/Valdis Goncarvos
Product Manager/PM Team Reviewer (31)
Registration Division (H7505C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 7/23/92
Toxicology Branch II, Section II,
Health Effects Division (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 7/23/92
Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 7/28/92
Chief, Toxicology Branch II/HFAS/HED (H7509C)

Registrant: Rohm & Haas Company
Chemical: 4,5-dichloro-2-n-octyl-3-(2H)-isothiazolone
Synonym: Kathon 287T Industrial Mildewcide
Caswell No.: 314B
Submission No.: S412458
DP Barcode: D175056
Identifying No.: 000707-EEU KATHON 287T
Case No.: 192535
MRID No.: 422149-03
Action Requested: Review the data and itemize the remaining data requirements.

Comment: The Registrant has submitted a 1991 study: Toxicity Study of RH-287 by Oral Administration to SD Rats for Four Weeks Followed by a Two-Week Recovery Period (Rohm and Haas Report No. 89RC-1033) to support the registration of Kathon 287T Industrial Mildewcide. The study has been reviewed, and the DER is appended.

Under the conditions of the study, exposure to RH-287 at dose levels of 20, 100, and 500 mg/kg via gavage once daily for 28 days resulted in death (high dose), decreased body weight/gain and food consumption (high dose), alteration of hematological/clinical

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chemistry/urinalysis parameters (mid and high dose), increased water consumption (mid and high dose), changes in organ weight (high dose), and histopathological lesions in the nonglandular stomach and small intestine (mid and high dose) that are consistent with effects on the liver, kidneys, adrenals, brain, gastrointestinal tract, as well as starvation/malabsorption. Several affected parameters (those in the stomach and adrenals) did not returned to normal values following two weeks of recovery. Possible target organs for RH-287 are the nonglandular stomach, the small and large intestine, adrenal gland, brain, kidneys, liver, gonads, and spleen.

The NOEL for a 4-week exposure period can be set at 20 mg/kg, the LEL at 100 mg/kg, based on increased water consumption, hematology/clinical chemistry changes, and histopathological lesions in the nonglandular stomach and small intestine.

This study is classified unacceptable, but it may be upgraded with the submission of information/data on the test material, formulations (stability of the suspensions, concentrations attained, homogeneity of the suspensions). The study does not satisfy any guideline requirement, but it does provide useful information on the toxicity of RH-287 following 28 days of exposure.

CONCLUSION

The current study on Kathon 287T is the only new study on this mildewcide submitted to TB II for review since the last update (TB II memo dated 5/15/90, copy appended) of the chemical. TB II is not aware of any additional toxicology studies on Kathon 287T. The data requirements (first-tier studies of the Antimicrobial Data Call-In) have not changed since the last update.

Tox Chem No. 314B Kathon 287T File Last Updated _____ Current Date 7/20/92

STUDY/LAB/STUDY #/DATE	MATERIAL	EPA MRID NO.	RESULTS: LD50, LC50, PIS, NOEL, LEL	TOX CATEGORY	CORE GRADE/DOC #
4-week oral - rat 8K280-E; R&H Report # 89RC-1033; Kashima Labs/Japan; 8/12/91	RH-287 (97.5%)	422149-03	Under the conditions of the study, exposure to RH-287 at dose levels of 20, 100, and 500 mg/kg via gavage once daily for 28 days resulted in death (high dose), decreased body weight/gain and food consumption (high dose), alteration on hematological/clinical chemistry/urinalysis parameters (mid and high dose), increased water consumption (mid and high dose), changes in organ weight (high dose), and histopathological lesions in the nonglandular stomach and small intestine (mid and high dose) that are consistent with effects on the liver, kidneys, adrenals, brain, gastrointestinal tract, as well as starvation/malabsorption. Several affected parameters (those in the stomach and adrenals) did not returned to normal values following two weeks of recovery. Possible target organs for RH-287 are the nonglandular stomach, the small and large intestine, adrenal gland, brain, kidneys, liver, gonads, and spleen. The NOEL for a 4-week exposure period can be set at 20 mg/kg, the LEL at 100 mg/kg, based on increased water consumption, hematology/clinical chemistry changes, and histopathological lesions in the nonglandular stomach and small intestine. This study is classified unacceptable, but it may be upgraded with the submission of information/data on the test material formulations (stability of the suspensions, concentrations attained, homogeneity of the suspensions). The study does not satisfy any guideline requirement, but it does provide useful information on the toxicity of RH-287 following 28 days of exposure.		Unacceptable 009636

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

REVIEWER

009636

MAY 15 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: KATHON 287T - TECHNICAL GRADE OF ACTIVE INGREDIENT

TO: JOHN LEE
PRODUCT MANAGER (31)
REGISTRATION DIVISION (H7505C)

FROM: LINDA L. TAYLOR, PH.D. Linda Lee Taylor 5/3/90
TOXICOLOGY BRANCH II, SECTION II
HEALTH EFFECTS DIVISION (H7509C)

THRU: K. CLARK SWENTZEL K. Clark Swentzel 5/4/90
SECTION II HEAD, TOXICOLOGY BRANCH II
HEALTH EFFECTS DIVISION (H7509C)

AND

MARCIA VAN GEMERT, PH.D. Marcia van Gemert 5/7/90
CHIEF, TOXICOLOGY BRANCH/HFAS/HED (H7509C)

REGISTRANT: ROHM ABD HAAS COMPANY
CHEMICAL: 4,5-DICHLORO-2-N-OCTYL-3(2H)-ISOTHIAZOLONE
SYNONYM: KATHON 287T
PROJECT NO.: 0-0921
CASWELL NO.: 314B
RECORD NO.: 257966
IDENTIFYING NO.: 707-EEU
MRID NO.: NOT APPLICABLE
ACTION REQUESTED: TECHNICAL FOR A NEW CHEMICAL; WILL DATA BASE SUPPORT USE?

COMMENT: THE COMPANY IS APPLYING FOR A PESTICIDE REGISTRATION FOR KATHON 287T. THE LABEL STATES THAT KATHON 287T IS FOR USE ONLY IN THE FORMULATION OF INDUSTRIAL MILDEWICIDES, THE ACTIVE INGREDIENT BEING 4,5-DICHLORO-2-N-OCTYL-3(2H)-ISOTHIAZOLONE (93.5%). INCLUDED ALSO IN THE PACKAGE IS THE CONFIDENTIAL STATEMENT OF FORMULA AND AN ATTACHMENT II, WHICH INCLUDES A LIST OF TOXICOLOGY DATA ON WHICH THE COMPANY IS RELYING.

KATHON 287T IS DESCRIBED IN ATTACHMENT I (TECHNICAL GRADE OF THE ACTIVE INGREDIENT - KATHON 287T) AS A TAN TO BROWN SOLID AT ROOM TEMPERATURE, WHICH IS COMPRISED OF APPROXIMATELY 97.6% ACTIVE INGREDIENT AND 2.4% MANUFACTURING BY-PRODUCTS AND PROCESS SOLVENT. THE REGISTRANT STATES THAT THERE ARE NO INTENTIONS AT THE PRESENT TIME TO COMMERCIALIZE KATHON 287T AS A FORMULATION USE PRODUCT, A STATEMENT THAT IS IN DISAGREEMENT WITH THE SUBMITTED LABEL. THE LABEL ALSO LISTS THE PERCENTAGE OF A.I. AS 93.5%.

THE TOXICOLOGICAL DATA AVAILABLE (PREVIOUSLY SUBMITTED TO THE AGENCY), WHICH ARE BEING RELIED ON ARE STUDIES PERFORMED ON FORMULATIONS CONTAINING VARIOUS AMOUNTS OF THE ACTIVE INGREDIENT WITH AND WITHOUT THE SOLVENT [redacted]. THESE FORMULATIONS INCLUDE:

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

A) 32% A.I. BY WEIGHT [redacted] (CURRENT MANUFACTURING-USE PRODUCT AND END-USE PR[redacted]ORMULATION)
B) 35%A.I. AND 5% [redacted]-2-N-OCTYL-3(2H)-ISOTHIAZOLONE, 12-18% RELATED IMPURITIES, [redacted] THIS FORMULATION IS NO LONGER COMMERCIALLY PRODUCED.
C) FORMULATION B, ABOVE WITHOUT [redacted]00% A.I.

THE TOXICITY DATA AVAILABLE INCLUDE:

ACUTE ORAL

1) ACUTE ORAL STUDY IN RATS USING (A) FORMULATION; LD50 MALES 4.4 G/KG (EQUIVALENT TO 1.4 G/KG A.I.); LD50 FEMALES 2.6 G/KG (EQUIVALENT TO 0.84 G/KG A.I.).

2) ACUTE ORAL STUDY IN MALE RATS USING (B) FORMULATION; LD50 1 89 (1.46-2.61) G/KG.

THE REGISTRANT STATES THAT THE TOXICITY CATEGORY WOULD NOT BE EXPECTED TO CHANGE (FROM CATEGORY III TO II) IF THE ACTIVE INGREDIENT ITSELF WERE TESTED; THEREFORE, A WAIVER IS REQUESTED FOR THE ACUTE ORAL STUDY FOR KATHON 287T. TB II DOES NOT CONCUR. AN ACUTE ORAL STUDY IS REQUIRED ON THE TECHNICAL GRADE OF KATHON 287T.

ACUTE DERMAL

3) ACUTE DERMAL STUDY IN RABBITS USING (A); LD50 > 2.0 G/KG (EQUIVALENT TO >0.65 G/KG A.I.).

4) ACUTE DERMAL STUDY (MALES) 1.7 (1-4.5) G/KG OF (B).

THE REGISTRANT STATES THAT A 32.6% FORMULATION OF THE A.I. IN [redacted] WAS FOUND TO BE CORROSIVE IN A SKIN IRRITATION STUDY IN RABBITS; THEREFORE, BECAUSE OF THE CORROSIVITY OF THE A.I., THE REGISTRANT REQUESTS A WAIVER OF THE ACUTE DERMAL STUDY ON KATHON 287T.

TB II DOES NOT CONCUR. AN ACUTE DERMAL STUDY IS REQUIRED OF THE TECHNICAL GRADE OF KATHON 287T.

ACUTE INHALATION

5) ACUTE INHALATION STUDY IN RATS USING (B); 0.72 MG/L 4-HOUR WHOLE BODY EXPOSURE (EQUIVALENT TO 0.29 MG/L A.I.).

THE REGISTRANT STATED THAT A 90-DAY INHALATION STUDY WAS INITIATED IN 1989 AND WILL BE SUBMITTED UPON COMPLETION; HOWEVER, THE TEST MATERIAL WAS NOT IDENTIFIED.

AN ACUTE INHALATION STUDY IS REQUIRED ON THE TECHNICAL GRADE OF KATHON 287T

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

PRIMARY EYE IRRITATION

6) PRIMARY EYE IRRITATION STUDY IN RABBITS USING (A) SHOWED MATERIAL TO BE SEVERELY IRRITATING TO THE EYES; OCULAR EFFECTS PERSISTED 21 DAYS AFTER EXPOSURE.

A WAIVER IS REQUESTED DUE TO THE SKIN CORROSIVITY NOTED ABOVE.

TB II CONCLUDES THAT DUE TO THE OCULAR EFFECTS OBSERVED FOLLOWING EXPOSURE TO A 32% FORMULATION OF KATHON 287T, THIS STUDY CAN BE WAIVED FOR THE TECHNICAL GRADE, PROVIDED THE LABEL BEARS THE APPROPRIATE SIGNAL WORD FOR A TOXICITY CATEGORY I COMPOUND.

PRIMARY DERMAL IRRITATION

7) PRIMARY DERMAL IRRITATION STUDY ON (A) - CORROSIVE.

8) TEST ON (B) SHOWED IT TO BE SEVERELY IRRITATING; PRIMARY IRRITATION SCORE BETWEEN 5 AND 8.

REGISTRANT REQUESTED A WAIVER. TB II CONCLUDES THAT A WAIVER CAN BE GRANTED, PROVIDED THE LABEL BEARS THE APPROPRIATE SIGNAL WORD FOR A CORROSIVE COMPOUND.

DERMAL SENSITIZATION

9) DERMAL SENSITIZATION STUDY ON (C) SHOWED IT TO BE A SENSITIZER.

ALTHOUGH THE REGISTRANT DID NOT REQUEST A WAIVER FOR THE TECHNICAL INGREDIENT, A STUDY NEED NOT BE PERFORMED ON KATHON 287T PROVIDED THE LABEL LISTS IT AS A SENSITIZER.

21-DAY DERMAL

10) 21-DAY DERMAL STUDY ON (B) RESULTED IN SKIN IRRITATION BUT NO DETECTABLE SYSTEMIC EFFECTS WERE NOTED.

TERATOLOGY

11) TERATOLOGY STUDY ON (B; ANTI-FOULANT C-9211M) IN THE RAT - NOT TERATOGENIC UNDER CONDITIONS OF STUDY AT DOSES UP TO 112.4 MG/KG/DAY; FETOTOXIC NOEL = 11.2 MG/KG, WITH INCREASED INCIDENCES OF BENT RIBS NOTED AT 33.7 MG/KG; MATERNAL NOEL = 11.2 MG/KG, THE LEL, 33.7 MG/KG (EFFECTS OBSERVED NOT STATED).

12) TERATOLOGY STUDY ON (B) IN RABBITS DEMONSTRATED NO EMBRYOTOXIC OR DEVELOPMENTAL TOXICITY EFFECTS IN FETUSES AT DOSES OF 5 AND 25 MG/KG; DUE TO COMPOUND-RELATED ABORTIONS, INSUFFICIENT NUMBERS OF LITTERS AVAILABLE TO ASSESS DEVELOPMENTAL TOXICITY AT THE HDT (70 MG/KG); TENTATIVE NOEL OF 25 MG/KG FOR DEVELOPMENTAL TOXICITY.

MUTAGENICITY

13) CHO/HGRT GENE MUTATION STUDY ON (B); NO MUTATIONS INDUCED WITH OR WITHOUT METABOLIC ACTIVATION.

14) IN VITRO CYTOGENETIC ASSAY IN CHO CELLS ON (B); MATERIAL DID NOT INDUCE MUTATIONS WITH OR WITHOUT METABOLIC ACTIVATION.

15) MAMMALIAN CELL TRANSFORMATION ON (C) - DID NOT INDUCE ANY SIGNIFICANT LEVEL OF TRANSFORMING ACTIVITY IN C3H 10T 1/2 MOUSE CELL SYSTEM UNDER CONDITIONS OF STUDY.

THESE STUDIES CANNOT BE USED TO SUPPORT THE REGISTRATION OF THE TECHNICAL GRADE OF KATHON 287T, EXCEPT AS NOTED UNDER 6, 7, 8, AND 9 ABOVE. STUDIES ON THE TECHNICAL GRADE OF THE TEST MATERIAL ARE REQUIRED FOR REGISTRATION. THIS COMPOUND IS CONSIDERED AN ANTIMICROBIAL, AND AS SUCH IS SUBJECT TO THE DATA REQUIREMENTS FOR THOSE COMPOUNDS. THE STUDIES REQUIRED FOR THE TECHNICAL GRADE OF THE A.I. (4,5-DICHLORO-2-N-OCTYL-3(2H)-ISOTHIAZOLONE), IN ADDITION TO ACUTE ORAL, DERMAL, AND INHALATION STUDIES, ARE:

1) 82-3 90-DAY DERMAL OR, IN THE CASE OF A DERMALLY CORROSIVE CHEMICAL, A 90-DAY FEEDING STUDY ALONG WITH DATA ON THE RELATIVE EFFICIENCY OF THE UPTAKE OF THE CHEMICAL VIA BOTH ROUTES OF EXPOSURE;

2) 82-4 90-DAY INHALATION, IF THE A.I. IS A GAS AT ROOM TEMPERATURE OR IF THE USE RESULTS IN RESPIRABLE DROPLETS;

3) 83-3 TERATOGENICITY (ONE SPECIES) - A HIGH EXPOSURE MAY TRIGGER A STUDY IN A SECOND SPECIES;

4) MUTAGENICITY BATTERY.

THE RESULTS OF THE FIRST-TIER STUDIES (IN ADDITION TO EXPOSURE INFORMATION) MAY INDICATE THE NEED FOR ADDITIONAL TESTING (TIERS 2,3, AS DESCRIBED IN THE ANTIMICROBIAL DATA CALL-IN, COPY ATTACHED). THE REGISTRANT SHOULD BE REQUESTED TO SUBMIT MORE DEFINITIVE USAGE INFORMATION (E.G., CONCENTRATIONS USED, CORRELATION OF THE CONCENTRATION OF THE ACTIVE INGREDIENT WITH A SPECIFIC USE, DEFINITION OF BUILDING MATERIALS AND CONSTRUCTION PRODUCTS), AND NDEB SHOULD BE CONTACTED FOR A DETERMINATION OF WHAT EXPOSURE INFORMATION IS REQUIRED.

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Reviewed by: Linda L. Taylor, Ph.D Linda Lee Taylor 7/23/92
Tox. Branch II, Section II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 7/23/92
Tox. Branch II, Head Section II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 4-Week Feeding - rats TOX. CHEM. NO.: 195C

MRID NO.: 422149-03

TEST MATERIAL: RH-287

SYNONYMS: 4,5-dichloro-2-n-octyl-3-(2H)-isothiazolone; Kathon 287T Industrial Mildewcide

STUDY NUMBER: 8K280-E; Rohm & Haas Report # 89RC-1033

SPONSOR: Rohm and Haas, Japan/Rohm and Haas Company

TESTING FACILITY: Kashima Laboratories, Japan

TITLE OF REPORT: Toxicity Study of RH-287 by Oral Administration to SD Rats for Four Weeks Followed by a Two-Week Recovery Period

AUTHOR: K Takeda

REPORT ISSUED: August 12, 1991

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: Under the conditions of the study, exposure to RH-287 at dose levels of 20, 100, and 500 mg/kg via gavage once daily for 28 days resulted in death (high dose), decreased body weight/gain and food consumption (high dose), alteration on hematological/clinical chemistry/urinalysis parameters (mid and high dose), increased water consumption (mid and high dose), changes in organ weight (high dose), and histopathological lesions in the nonglandular stomach and small intestine (mid and high dose) that are consistent with effects on the liver, kidneys, adrenals, gastrointestinal tract, as well as starvation/malabsorption. Several affected parameters (those in the stomach and adrenals) did not returned to normal values following two weeks of recovery. Possible target organs for RH-287 are the nonglandular stomach, the small and large intestine, adrenal gland, kidneys, liver, gonads, and spleen.

The NOEL for a 4-week exposure period can be set at 20 mg/kg, the LEL at 100 mg/kg, based on increased water consumption, hematology/clinical chemistry changes, and histopathological lesions in the nonglandular stomach and small intestine.

Classification: Unacceptable. This study may be upgraded with the submission of information/data on the test material formulations (stability of the suspensions, concentrations attained, homogeneity of the suspensions). The study does not satisfy any guideline requirement, but it does provide useful information on the toxicity of RH-287 following 28 days of exposure.

A. MATERIALS:

1. Test Compound: RH-287; Description: not provided; Batch #: Lot # 12-SS-38-3; Purity: 97.5%.

2. Test Animals: Species: rat; Strain: Sprague-Dawley (SD) (SPF); Age: 5 weeks old at study start; Weight: males 147-164 g, females 108-134 g; Source: Charles River Japan, Inc..

3. Statistics: Differences in numerical data between the control and treated groups - Student's t-test; if the null hypothesis of the F test was not significant; if significant, Welch's t-test was used. Additionally, Armitage's X^2 test was used to assess differences of non-numerical data in the urinary paper tests.

B. STUDY DESIGN

1. Methodology: Forty males and 40 females were assigned (randomization procedure not mentioned) to one of four groups [0, 20, 100, or 500 mg/kg], each composed of 10 rats/sex/group, and administered the test material or vehicle (olive oil) via gavage (dosing volume: 5 mL/kg) once a day for 28 consecutive days. An additional twenty rats/sex were allowed a 14-day recovery period following administration of the test material or vehicle (10 rats/sex/group) for 28 days. The rats were housed 2/sex/cage. The animals were fed a standard laboratory pelleted chow Oriental MF (Oriental Yeast Co., Ltd./Tokyo). Both diet and tap water were available ad libitum. Appropriate amounts of the test material were measured accurately and suspended in olive oil warmed in a water bath (40° C); the formulations were prepared once or twice a week.

RESULTS

No information was provided regarding the homogeneity of the suspensions, but it was stated that chemical analysis confirmed that the test material remained stable for 14 days. No data on the analysis of the test material formulations were provided.

2. Clinical Observations: The animals were observed daily (physical signs and behavior) for evidence of any systemic reaction to treatment or ill-health. Individual body weights were recorded at study initiation and weekly thereafter. Food consumption was calculated as the total amount of food consumed per cage divided by the number of rat-days. Rat-days were calculated as the total number of living rats in a cage summed for each day of the week. Water consumption (calculated as the total amount of water consumed in each cage divided by the number of rat-days) was measured weekly from week 3 on,

since the high-dose rats were said to have displayed an increase (no indication as to how this was determined).

RESULTS

Survival and Clinical Observations

Three high-dose females were found dead on day 4 of treatment, and one mid-dose female died accidentally on day 22. There were no toxic signs observed that could be attributed to treatment in either sex at the low-dose level. Salivation was the only clinical sign noted in both sexes at the mid-dose level. Males at the high-dose level displayed anal staining (probably caused by diarrhea), reduced spontaneous movements, salivation, hypothermia, and abdominal distention. Salivation was not observed during the recovery period, although reduced spontaneous movements and abdominal distention persisted for a few days following termination of treatment. In the high-dose females, similar findings were noted (reduction in spontaneous movements, salivation, abdominal distention, staining of fur, and hypothermia), and loss of hair from the abdomen and back, cyanosis, reddish lacrimation, and gasping were observed. NOTE: Although diarrhea is mentioned in the text as a factor in explaining some of the effects noted, it is not listed in the table of clinical signs, nor is the incidence per group presented.

Body Weight and Food Consumption

Body weight was decreased after one week of treatment in both sexes at the high-dose level, with males more severely affected than females. The high-dose males continued to display decreased body weight throughout the study and during the recovery period, while the females displayed similar body weights to the controls from week 2 on. Body weights of the low- and mid-dose animals of both sexes were comparable to or greater than the control groups throughout the study. Body-weight gains were lower in the high-dose males throughout the study, but only at week one for the high-dose females. The authors did not provide any analysis of body-weight gains. The overall body-weight change as a % and in grams (calculated by TB II; no statistical analysis) are shown below.

Mean Body Weight (% of control)

Interval/mg/kg	20	100	500
MALES			
0	101	101	100
1	99	99	78***
2	98	99	78***
3	98	99	79***
4	98	97	78***
5	-	-	84***
6	-	-	88***

Interval/mg/kg	20	100	500
FEMALES			
0	100	103	102
1	100	105	96*
2	98	110	101
3	100	111	104
4	101	111	104
5	-	-	103
6	-	-	104

* $p<0.05$; *** $p<0.001$

Mean Body-Weight Change (g)

Interval/mg/kg	0	20	100	500
MALES				
0-1	65	61	61	16
0-2	139	133	134	75
0-3	192	185	185	118
0-4	236	229	224	150
0-5	265	-	-	200
0-6	296	-	-	243
FEMALES				
0-1	27	27	31	19
0-2	53	50	66	52
0-3	73	73	90	79
0-4	89	91	108	96
0-5	101	-	-	106
0-6	117	-	-	125

Body-Weight Change (%)

Weeks/mg/kg	0	20	100	500
MALES				
0-4	154	149	145	98
FEMALES				
0-4	74	76	87	79

Food consumption was decreased during the first week at all dose levels in males and at the high-dose level in females. Thereafter, males at the low- and mid-dose levels and females at the low-dose level displayed intakes that were comparable to their respective controls. The high-dose males displayed decreased intakes during the third and fourth weeks, but an intake similar to the control during the second week. The mid- and high-dose females displayed increased intakes compared to the control value from week 2 on. Increased intakes were observed in the high-dose animals of both sexes during the first week of the recovery period.

Mean Food Consumption (% of control)

week/mg/kg	20	100	500
MALES			
1	95*	94*	58***
2	105	104	95
3	96	97	87***
4	97	95	88*
5	-	-	117**
6	-	-	103
FEMALES			
1	101	101	79***
2	99	114***	121***
3	99	113**	120***
4	104	112*	114**
5	-	-	114*
6	-	-	95

* p<0.05; ** p<0.01; *** p<1.001

3. Blood Analyses

Hematology: Blood samples were obtained from all animals that survived treatment. It was not stated whether food was withheld prior to sacrifice and sample collection (from the vena cava under anesthesia). The CHECKED (X) parameters were examined.

X		X	
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		Red cell morphology
X	(Prothrombin time)		
X	(Activated partial thromboplastin time)		
	Nucleated erythrocytes normoblasts		

RESULTS

There were several treatment-related effects observed on the measured parameters in both sexes. There was a dose-related decrease in hemoglobin, MCV, and MCH in males, and males at the high-dose level also displayed decreases in hematocrit, MCHC and lymphocyte count and increases in reticulocyte counts, segmented neutrophils, and prothrombin time compared to the control values. Females displayed similar changes in these parameters, although statistical significance was attained only in the high-dose females for hemoglobin, MCH, and MCHC compared to control values.

MALE HEMATOLOGICAL FINDINGS♥

Parameter/Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
HGB	14.2	13.9	13.3*	12.3***
HCT	41.5	41.5	41.3	37.7***
MCV	51.1	51.7	48.6*	46.1***
MCH	17.5	17.3	15.7**	15.1***
MCHC	34.3	33.6	32.3**	32.7**
Retic.	21	26	30	29*
Seg. neutroph.	7	7	9	19**
Prothr. time	13.5	13.5	13.7	13.9**
Lymphocytes	91	89	88	76***

♥ HBG: g/dL; HCT/MCHC/Retic/seg. neu./lymph.: %; MCV:μM^3; MCH: PG; PT: sec.;

FEMALE HEMATOLOGICAL FINDINGS♥

Parameter/Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
HGB	14.7	14.0	14.6	13.0**
HCT	42.3	41.7	43.6*	40.1
MCV	50.8	50.6	50.9	50.4
MCH	17.7	17.0	17.0	16.3**
MCHC	34.7	33.7	33.5	32.4**
Retic.	14	17	13	18
Seg. neutroph.	5	5	5	11
Prothr. time	14.2	14.2	14.1	14.2
Lymphocytes	92	93	92	85

♥ HBG: g/dL; HCT/MCHC/Retic/seg. neu./lymph.: %; MCV:μM^3; MCH:PG; PT: sec.;

Reticulocyte counts remained increased and MCH and PT decreased in the high-dose males following the recovery period. APTT was significantly decreased following recovery in males. In the high-dose females, MCHC remained decreased following recovery, and there was an increase in hematocrit compared to the control value.

Clinical Chemistry: Blood samples were obtained as stated above. The CHECKED (X) parameters were examined.

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

X
Other:
- [ ] Albumin
- [X] Blood creatinine
- [X] Blood urea nitrogen
- [X] Cholesterol
- [ ] Globulins

X	Sodium	X	Glucose
	Iron		Phospholipids
Enzymes			Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum Protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
	Creatine kinase (CK)		Lipids, total
	Lactate dehydrogenase (LAD)		Triiodothyronine, total T3
X	Serum alanine aminotransferase		
X	Serum aspartate aminotransferase		
	Gamma glutamyl transferase (GGT)		
X	Gamma glutamyl transpeptidase (GTP)		
	Glutamate dehydrogenase (GLDH)		
	Ornithine carbamyltransferase (OCT)		
	Serum protein electrophoresis*		
	Thyroxine, total T4		

RESULTS

There was a dose-related increase in GOT, inorganic phosphorus, and A/G ratio and a dose-related decrease in glucose and total protein in the males following treatment. Other changes displayed by the high-dose males were an increase in GPT and sodium values compared to the control value. High-dose females displayed increases in GOT, total cholesterol, and inorganic phosphorus and decreases in glucose, total protein, and chloride compared to the control value. There was an increase in inorganic phosphorus at each dose level in females, but the increase was not dose-related. Additionally, there was a dose-related decrease in chloride values in the females.

MALE CLINICAL BIOCHEMISTRY FINDINGS♥

Parameter/Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
GOT	89	91	103*	115**
GPT	40	39	45	51**
Proteins TP	6.18	6.14	5.82*	5.41***
A/G ratio	1.36	1.34	1.55**	1.66***
Glucose	170	170	155**	132***
Inorganic P	7.8	8.1	8.2*	8.5*
Na	144	143	144	145***
Cl	104	103	103	102
T. chol.	63	60	59	71

♥ GOT/GPT [U/L]; TP [g/dL]; GLU/CHOL/IP/CA [MG/DL]; NA/CL [MEQ/L]

FEMALE CLINICAL BIOCHEMISTRY FINDINGS♥

Parameter/Dose	0 ppm	20 ppm	100 ppm	500 ppm
GOT	89	99	100	114**
GPT	30	34	29	36
Proteins TP	6.24	6.03	5.96	5.69**
A/G ratio	1.68	1.60	1.69	1.67
Glucose	169	174	162	144**
Inorganic P	8.0	8.8*	8.7*	9.3**
Na	144	144	143	145
Cl	105	104	103*	102**
T. chol.	56	60	59	67**

♥ GOT/GPT [IU/L]; TP [g/dL]; GLU/CHOL/IP/CA [MG/DL]; NA/CL [MEQ/L]

Following the recovery period, total protein values remained decreased and the A/G ratio, inorganic phosphorus, and sodium values remained increased in the males compared to the control values. Additionally, alkaline phosphatase and potassium were increased in males compared to the control values. In females, total cholesterol and inorganic phosphorus values remained increased compared to the control values. All other parameters in both sexes were comparable to their respective control values.

4. Urinalysis: Urine samples were collected from each animal after 3 weeks of treatment and after one week of the recovery period. The analyses (marked with an ♦) were performed on individual fresh urine samples. Urine samples were collected in individual metabolic cages overnight (≈ 16.5 hours; under standard food and water intake) and analyzed for the parameters marked with an ♣. The CHECKED (X) parameters were examined.

X		X	
	Appearance	X	Glucose♦
X	Volume♣	X	Ketones♦
X	Specific gravity♣	X	Bilirubin♦
X	pH♦	X	Blood♦
	Sediment (microscopic)		Nitrate
X	Protein♦	X	Urobilinogen♦
	Osmolality		Color
X	Sodium♣	X	Chloride♣
X	Potassium♣		

RESULTS

In males, there was a dose-related increase in urine volume and bilirubin levels and acid urine following treatment.

Additionally, the high-dose males displayed decreases in specific gravity, sodium, and potassium compared to the control values. Females displayed decreases in urobilinogen at all dose levels compared to the control value, and the mid- and high-dose females displayed acid urine, increased protein and chloride values, and decreased ketones compared to the control values.

Urinalysis Data

Parameter/Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
MALES				
pH	7.88	7.8	6.55	6.58
volume	9.2	10.8	11.9	11.7
bilirubin	-	+	3+	7+
specific gravity	1.076	1.063	1.064	1.056
sodium	1.40	1.21	1.61	0.77
potassium	3.06	2.81	3.32	2.57
FEMALES				
urobilinogen♣	75/25	10/90	10/90	18/82
pH	7.35	6.95	6.55	6.09
protein♦	36.5(25)	55.0(10	143(10)	94.7(0)
volume	7.3	6.2	7.9	18.6
chloride	1.22	1.06	1.28	1.58
ketone bodies♦	6.7(10)	9.4(10)	7.0(0)	7.2(40)

* p<0.05; ♦ (% with negative value); ♣ % with a value of 1/% with a value of 0.1; ♦ (% with negative value or trace)

Following the recovery period, males displayed increased urinary chloride, and urine volume remained elevated. There were no significant differences observed in the females.

NOTE: Water consumption was measured during week 4 of the study and each week of the recovery period. Significant increases were observed in the mid- and high-dose levels of both sexes at week 4, and both sexes displayed increased consumption during the first week of recovery; females also displayed an increase in the second recovery week.

Water Consumption (mL/rat/day)

Period/Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
MALES				
Dosing	32.0	30.8	36.8*	48.9***
Recovery 1	33.1	-	-	44.8***
Recovery 2	35.9	-	-	38.1
FEMALES				
Dosing	21.9	23.2	25.2*	45.5***
Recovery 1	23.1	-	-	33.3*
Recovery 2	36.3	-	-	33.0*

6. Gross Pathology: All animals were subjected to a full macroscopic examination at sacrifice (no information on whether animals were fasted overnight was provided). The following organs were weighed: kidneys, liver, testes/ovaries, brain, spleen, and adrenals.

RESULTS

In the 3 females that died on test, atrophy of the thymus and atrophy of the spleen with discoloration were observed. Additionally, two of these females displayed edema of the nonglandular stomach, and the other females displayed hemorrhage of the mucosa of the glandular stomach and fibrous adhesions of the liver and gastric serosa.

In the rats sacrificed after treatment, gross findings were observed only in animals of the high-dose group. One rat of each sex displayed no gross lesions. The thickening of the wall of the stomach, small and large intestine was observed in the same 4 males; the female with thickening of the stomach also displayed thickening in the small and large intestine, and the 2 with thickening in the large intestine displayed it in the small intestine as well. Five males displayed atrophy of the liver only. A cyst was observed in the kidney of one of 9 high-dose recovery females, and one high-dose recovery male of the 10 displayed discoloration of the kidney.

Rats Displaying Macroscopic Finding - Dosing phase

Gross Finding/sex	MALE (10)*	FEMALE (7)*
Atrophy of liver	5	0
Atrophy of spleen	0	2
Thickening of gastric wall	4	1
thickening of sm. intestinal wall	4	7
thickening of lg. intestinal wall	4	2

* (# examined)

Males at the high-dose level displayed significant decreases in absolute brain, liver, kidney, spleen, and testes weight, and an increase in absolute adrenal weight. Relative (to-body-weight) brain, adrenal, and testes weights were increased, and relative liver weight was decreased in the high-dose males. Mid-dose males also displayed decreased relative liver weight. Increased absolute adrenal weight and decreased absolute brain weight were observed at the high-dose level in females, and the mid-dose females displayed increased absolute liver weight. Relative adrenal weight was increased in the high-dose females and relative brain weight was decreased in the mid-dose females. The majority of the organ-weight changes observed in the high-dose males can be attributed to decreased body weight. With regard to the decrease in brain weight, which was observed in both sexes, decreased body-weight would not be expected to affect brain weight, especially over the short time-frame of this study, and examination of the individual brains indicates that the decrease was displayed in most of the animals. For example, the percent of males with brain weights of less than 2 grams was 10% in the controls, 0% in the low, 10% in the mid, and 70% in the high dose groups.

In females, the % ≤ 1.85 grams was 20%, 20%, 20%, and 75%, respectively. With regard to the adrenal weight, increases in both the absolute and relative weight are considered related to treatment.

Following the recovery phase, high-dose males displayed decreased absolute brain, liver, and kidney weights, and increased relative adrenal weight. Absolute ovarian weight was increased in the high-dose females following the recovery phase; relative organ weights were comparable to those of the control females.

Absolute Organ Weight (g or mg)

Organ/Dose (mg/kg)	0	20	100	500
MALES				
brain	...12	2.09	2.08	1.98***
liver	7.48	17.19	15.90	12.39***
kidneys	2.88	2.91	2.72	2.23***
adrenals	58.7	53.4	57.7	72.4**
spleen	0.80	0.77	0.79	0.56***
testes	2.96	2.93	3.05	2.72*
FEMALES				
brain	1.93	1.91	1.93	1.84*
liver	9.19	9.26	10.43*	9.51
kidneys	1.69	1.74	1.81	1.80
adrenals	68.3	65.6	68.3	83.5*
spleen	0.51	0.48	0.55	0.45
ovaries	87.7	80.6	92.3	91.1

Relative Organ Weight (% of body wt)♦

Organ/Dose (mg/kg)	0	20	100	500
MALES				
brain	0.54	0.55	0.55	0.68***
liver	4.46	4.48	4.20*	4.20*
adrenals	15.0	14.0	15.3	24.7***
testes	0.76	0.77	0.81	0.93***
FEMALES				
brain	0.93	0.91	0.84**	0.89
adrenals	32.4	31.3	29.6	40.7*

♦ adrenal x 10^3; * p<0.05; ** p<0.01; *** p<0.001

Recovery Phase Absolute Organ Weight (g or mg)

Organ/Dose (mg/kg)	0	500
MALES		
brain	2.24	2.10**
liver	17.62	14.74**
kidneys	3.13	2.87**
adrenals	60.8	62.6
FEMALES		
ovaries	85.8	98.7*

Recovery Phase Relative Organ Weight (% bw x 10^3))

Organ/Dose (mg/kg)	0	500
MALES adrenals	13.6	15.8*

* p<0.05; ** p<0.01; *** p<0.001

7. Histopathology: The following organs/tissues (CHECKED (X)) were preserved from all rats. The heart, liver, spleen, kidneys, and adrenals of the control and high-dose animals were examined microscopically; since treatment-related lesions were found in the spleen and adrenals, these organs were examined in all animals. Additionally, at necropsy, lesions were observed in the stomach and intestinal tract, which were considered treatment-related, and these were examined in all animals. The spleen, adrenals, stomach, and intestinal tract were examined in all recovery animals, and the liver of one high-dose female sacrificed at 28 days and the kidneys of one male and one female at the high-dose were examined microscopically, since abnormal findings were recorded in these organs at necropsy.

X	Digestive system
	Tongue
	Salivary glands
X	Esophagus
X	Stomach
X	Duodenum
X	Jejunum
X	Ileum
X	Cecum
X	Colon
X	Rectum
X	Liver
	Gall bladder
	Pancreas
	Respiratory
	Trachea
X	Lung
	Nose
	Pharynx
	Larynx

X	Cardiovasc./Hemat.
	Aorta
X	Heart
X	Bone marrow♥
	Lymph nodes
X	Spleen
	Thymus
	Urogenital
X	Kidneys
X	Urinary bladder
X	Testes
	Epididymides
	Prostate
	Seminal vesicle
X	Ovaries
	Uterus
	Vagina
	Coagulating gland

♥femur

X	Neurologic
X	Brain
	Periph. nerve
	Spinal cord
X	Pituitary
X	Eyes
	Glandular
X	Adrenal gland
	Lacrimal gland
	Mammary gland
X	Parathyroids
X	Thyroids
	Other
	Bone♥
	Skeletal muscle
	Skin
	All gross lesions

RESULTS

Female rats that died on test: Atrophy of the thymus (predominantly due to loss of the cortical lymphocytes by destruction, which was accompanied by a decrease of lymphocytes in the medulla) and spleen (characterized by atrophy of the white pulp and a decrease of hematopoietic

cells) and necrosis of the nonglandular stomach mucosa (consisted of coagulation necrosis of the mucosa with marked infiltration of neutrophils) were observed in all three females that died on test. Additionally, in the tunica propria mucosae of the nonglandular stomach, proliferation of fibroblasts, edema, and the exudation of a fibrin-like substance was observed. Hemorrhage was observed in the adrenals (zona fasciculata bilaterally) of two of these females. Capsulitis of the liver was observed in one of these females, which was characterized by infiltration of neutrophils and fibrous thickening of the capsule and considered as extension of the lesions of the nonglandular stomach.

Terminal sacrifice: Treatment-related findings (see table below) included an increase of granulocytes in the spleen, hyperplasia of the mucosa of the nonglandular stomach, granulation tissue in the tunica propria mucosae of the nonglandular stomach, hyperplasia of the mucosa of the small intestine, and an increase in the lipid content of the adrenal zona fasciculata. Hyperplasia of the mucosa in the nonglandular stomach consisted of thickening of the stratified squamous epithelium with hyperkeratosis. Granulation was characterized by the appearance of giant cells, the infiltration of lymphocytes, the proliferation of fibroblasts, and the neoformation of capillaries. Mucosal hyperplasia in the small intestine consisted mainly of the elongation of the villi in the duodenal mucosa. The change displayed in the adrenals consisted of clear cytoplasm produced by numerous minute vacuoles containing neutral fat that stained positively with Sudan III.

Male Histopathological Lesions

Phase	Treatment Period				Recovery	
Dose (mg/kg)	0	20	100	500	0	500
Lesion/n=	10	10	10	10	10	10
Spleen						
↑ granulocytes	0	0	0	4	0	0
Stomach						
nonglandular area:						
hyperplasia of mucosa	0	0	5	10	0	5
granulation	0	0	0	3	0	6
Small intestine						
hyperplasia of mucosa	0	0	4	10	0	0
Adrenals						
↑ lipid zona fasciculata	0	0	1	9	0	3
Heart						
myocardial degeneration	0	0	0	1	0	0

Female Histopathological Lesions

Phase	Treatment Period				Recovery	
Dose (mg/kg)	0	20	100	500	0	500
Lesion/n=	10	10	9	8	10	9
Spleen						
↑ granulocytes	0	0	0	3	0	0
Stomach						
nonglandular area:						
hyperplasia of mucosa	0	0	9	8	0	2
granulation	0	0	0	4	0	3
Small intestine						
hyperplasia of mucosa	0	0	5	7	0	0
Adrenals						
↑ lipid zona fasciculata	0	0	0	5	0	5

Recovery animals: The findings in the nonglandular stomach and adrenals were also observed in the recovery animals.

Histological Findings

lesion/dose	0 mg/kg	500 mg/kg
MALES n=	10	10
Stomach		
nonglandular area		
hyperplasia of mucosal epithelium	0	5
granulation	0	6
Adrenals		
lipid increase/fascicular zone	0	3
Kidneys		
cyst	0	1
FEMALES n=	10	9
Stomach		
nonglandular area		
hyperplasia of mucosal epithelium	0	2
granulation	0	3
Adrenals		
lipid increase/fascicular zone	0	5
Kidneys		
cyst	0	0

DISCUSSION

The objective of the study was to determine the toxicity of RH-287 by "serial observations of the functional and morphological changes in animals receiving the substance daily for four weeks." Three females of the high-dose group (500 mg/kg) died on test. Clinical signs attributed to treatment include salivation, reduced spontaneous movements, anal staining, hypothermia, and abdominal distention. Body weight/change were decreased at the high-dose in both sexes after one week, with males affected more severely than females; only the males continued to display decreases in body weight throughout the study. Food intake was decreased at all dose levels in males and in females at the high-dose level during the first week; thereafter, high-dose males displayed

decreased intake during weeks 3 and 4. During recovery, the high-dose animals of both sexes displayed increased intakes compared to the control values during the first week. Water consumption was increased in both sexes at the mid- and high-dose levels during week 4 of treatment and the recovery phase. Several hematology, clinical chemistry, and urinalysis parameters were affected by treatment in both sexes at the mid- and high-dose levels, which are consistent with excessive fluid intake, malabsorption/starvation, and liver/kidney/adrenal/gastrointestinal effects. Treatment-related increases in adrenal weight and treatment-related decreases in brain weight were observed in the high-dose animals of both sexes. Histologically, mucosal hyperplasia of the nonglandular stomach and small intestine, increased fat droplets in the cells of the zona fasciculata of the adrenals, increased granulocytic series hemopoietic cells in the spleen, and formation of granulation tissue in the tunica propria of the nonglandular stomach were observed in survivors at the mid- and high-dose levels, and atrophy of the liver and spleen, and thickening of the walls of the stomach, small intestine and large intestine were observed in those that died on test. Possible target organs for RH-287 are the nonglandular stomach, the small and large intestine, adrenal gland, kidneys, liver, brain, and spleen. Additionally, due to the increase (absolute and relative) in ovarian weight in the high-dose females that was observed following recovery, the ovaries/ gonads may be target organs also.

CONCLUSION

Under the conditions of the study, exposure to RH-287 at dose levels of 20, 100, and 500 mg/kg via gavage once daily for 28 days resulted in death (high dose), decreased body weight/gain and food consumption (high dose), increased water consumption (mid and high dose), alteration of hematological/clinical chemistry/urinalysis parameters (mid and high dose), organ weight changes (high dose), and histopathological lesions in the nonglandular stomach and small intestine (mid and high dose) that are consistent with effects on the liver, kidneys, adrenals, gastrointestinal tract, as well as starvation/ malabsorption. Several affected parameters (those in the stomach and adrenals) did not returned to normal values following two weeks of recovery. The NOEL for a 4-week exposure period can be set at 20 mg/kg, the LEL at 100 mg/kg, based on increased water consumption, hematology/clinical chemistry changes, and histopathological lesions in the nonglandular stomach and small intestine.

This study is classified unacceptable, but it may be upgraded with the submission of information/data on the test material formulations (stability of the suspensions, concentrations attained, homogeneity of the suspensions). The study does not

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satisfy any guideline requirement, but it does provide useful information on the toxicity of RH-287 following 28 days of exposure.