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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 707-ERE;ERN;ERR/7F3476;7H5524. RH-3866 (Rally™) Fungicide. Application for Registration and Petition for Permanent Tolerances on Apples, Grapes, Processed Commodities, Meat, Milk and Eggs.

Tox. Chem. No. 723K
Project No. 7-0519

TO: Lois Rossi, PM #21
Fungicide-Herbicide Branch
Registration Division (TS-767c)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

THROUGH: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

Budd 2/9/88

Record Nos. 185801/185802/(185800)/185797/185799

Background and Request:

Rohm and Haas has filed an application for registration of a new pesticide, RH-3866 Technical, and two end-use products, Rally™ 40W fungicide and Rally™ 60DF fungicide, all containing the active ingredient, myclobutanil. Included with the application is a petition for permanent tolerances for residues of the active ingredient and its toxicologically significant metabolites in or on raw agricultural commodities (apples, grapes), processed commodities, meat, milk, and eggs. Several petitions for this pesticide have been previously reviewed by the Toxicology Branch (TB). These petitions included applications for temporary tolerances and Experimental Use Permits (EUP's). In the present petition, the following permanent tolerances were requested:

	Commodity:	ppm
Apples:	Whole fruit	0.5 ppm
	Wet Pomace	1.0 ppm
	Dry Pomace	5.0 ppm

1

	Commodity:	ppm:
Grapes:	Whole Fruit	1.0 ppm
	Wet Pomace	2.0 ppm
	Dry Pomace	10.0 ppm
	Raisins	10.0 ppm
	Raisin Waste	25.0 ppm
Meat and Meat By-Products (except liver)		0.04 ppm
Liver (cattle, goats, hogs, horses or sheep)		0.5 ppm
Milk		0.1 ppm
Eggs		0.04 ppm

Response:

The Toxicology Branch (TB) cannot recommend granting the application for registration of these products and the establishment of tolerances for residues until the toxicology data requirements are completed. The following pages discuss the data requirements that need to be completed before registration of the products.

The following toxicity studies are recommended to be submitted in support of the proposed registration and tolerances. Those recommendations that have been satisfied are indicated:

	Required	Satisfied
Technical Product		
Acute oral LD_{50} - rat	Yes	Yes
Acute dermal LD_{50}	Yes	Yes
Acute inhalation LC_{50}	Yes	Yes (Comment 1)
Primary eye irritation	Yes	Yes
Primary dermal irritation	Yes	Yes
Dermal Sensitization	Yes	No (Comment 2)
90-day feeding - rodent	Yes	Yes
90-day feeding - nonrodent	Yes	Yes
21-day dermal	Yes	Yes (Comment 3)
Chronic feeding		
rodent	Yes	Yes
nonrodent	Yes	Yes

Technical (cont.)	Required	Satisfied
Oncogenicity		
Mouse	Yes	No (Comment 4)
Rat	Yes	No (Comment 4)
Teratology		
Rat	Yes	Yes
Rabbit	Yes	Yes
Reproduction	Yes	Yes
Gene mutation	Yes	Yes
Dominant lethal	Yes	No (Comment 5)
Chromosomal aberration	Yes	Yes
Other genotoxic effects	Yes	Yes
Metabolism	Yes	Yes (Comment 6)
Dermal Penetration	No	No (Comment 7)
Rally® 40 WP		
Acute oral LD_{50} - rat	Yes	Yes
Acute dermal LD_{50}	Yes	Yes
Acute inhalation LC_{50}	Yes	Yes
Primary eye irritation	Yes	Yes
Primary dermal irritation	Yes	Yes
Dermal sensitization	Yes	No (Comment 8)
21-Day dermal	Yes	Yes
Rally® 60DF		
Acute oral LD_{50} - rat	Yes	Yes
Acute dermal LD_{50}	Yes	Yes
Acute inhalation LC_{50}	Yes	No (Comment 9)
Primary eye irritation	Yes	Yes
Primary dermal irritation	Yes	No (Comment 9)
21-day dermal	Yes	Yes (Comment 3)
Dermal sensitization	Yes	No (Comment 9)

1. In light of the facts that the acute inhalation study on the 40% wettable powder indicated that it was of low toxicity via the inhalation route and that the Technical Product is a solid with a low vapor pressure, TB is waiving the requirement for an acute inhalation study on the Technical Product for this particular registration application.

2. The requirement for a dermal sensitization study for registration of technical RH-3866 as a manufacturing use product will be waived if dermal sensitization studies on the two end-use products, Rally™ 40WP and Rally™ 60DF, are negative for sensitizing properties.

3. In a memorandum dated February 12, 1986 from J.E. Harris to H. Jacoby, Dr. Harris stated that a 21-day dermal study on RH-3866 should be conducted with particular attention given to any possible testicular effects. Since acceptable 4-week dermal studies on Rally™ 40WP and another formulation of RH-3866 (2EC) have now been evaluated and found to be negative for testicular effects, the requirement for a 21-day dermal study is considered by the Toxicology Branch to have been satisfied.

4. The oncogenicity studies conducted on rats and mice were classified as Core Supplementary because the MTD may not have been reached in either case. This classification, however, is subject to change pending consideration of this matter by an ad hoc TB committee which will meet in the near future to decide whether dosage levels employed in these oncogenic studies were or were not acceptable and whether either or both of the studies may have to be repeated.

   It is noted that EPA staff met with representatives from Rohm and Haas on January 22, 1988 to discuss this issue. (See memorandum from Pamela M. Hurley to L. Rossi/L. Schnaubelt, dated 1/27/88 for a summary of this meeting).

5. The dominant lethal study was well conducted and excellently presented. However, the study is classified as unacceptable because positive control data were lacking. The study will be upgraded to acceptable upon receipt of appropriate historical positive control data from the period this test was performed in the same strain of rats.

6. In comparing the rat metabolism data with the residues in apples, grapes, milk, liver and eggs, it appears that in the toxicity studies, the rats were exposed to significant quantitities of metabolites that are identical to those found in the products listed above. Therefore, no further toxicity testing is required for the metabolites. For additional information and discussion relating to plant and animal metabolites and regulable residues, see the memorandum from Pamela M. Hurley to Maxie Jo Nelson, dated 12/17/87.

7. The dermal penetration study is unacceptable because the failure to perform an analysis of the application site skin and residue in the carcass makes it impossible to verify recovery. It is recommended that the Registrant analyse the application site skin and the carcasses of

the animals used in the study in order to complete the material balance determination. Although this study is not required (at this time) to support the requested registrations and tolerances, it would be highly advisable for the applicant to repair the deficiencies in this study in order that it might be used in the calculation of a margin of safety (MOS) for testicular effects (see comment 12 in this memorandum).

8. In a memorandum dated April 22, 1987 from P. Hurley to L. Rossi, TB stated that a dermal sensitization study will be required for the registration of the 40WP formulation.

9. The required acute toxicity data on the 60DF formulation is being addressed by the Registration Division (see memorandum from D. Graham to L. Rossi, dated January 13, 1987).

10. The labels on the three products should be changed as follows:

a) The technical product is in Toxicity Category I for eye irritation. In light of this, the label should be changed from Warning to Danger. In addition, a statement should be added that this chemical is severely irritating to the eyes and that goggles and/or a face shield should be worn.

b) The 40 WP formulation is in Toxicity Category II for eye irritation. Therefore, the label should be changed from Caution to Warning and appropriate warnings and precautionary statements for a Toxicity Category II eye irritant should be added to the label. The label may change again depending upon the results from the dermal sensitization study.

c) To the label for the 60 DF formulation should be added appropriate warnings and precautionary statements for a Toxicity Category II eye irritant. Since EPA has no data for acute inhalation, dermal irritation and dermal sensitization, the label may change again depending upon the results from these studies.

d) It should be noted that the labels for the 40 WP and 60 DF formulations presently require the user to wear mid-forearm to elbow-length chemical-resistant gloves, hat, long-sleeved shirt and long pants when mixing, loading and applying the product. This statement (which is <u>not</u> required by the results of <u>acute</u> toxicity studies) was added to the label by the Registrant in view of the testicular lesions observed in rats in the reproduction and chronic studies. Although TB considered this statement appropriate for the EUP

program (see memorandum from Jane E. Harris to Henry M. Jacoby, dated 2/11/86), the question of whether it should remain, or be removed or be modified for the purpose of conditional or full registration cannot be answered at this time. Prior to making a determination on this matter, it will be necessary to consider, among other things, the MOS(s) for testicular effects for users of these products (see comment 12 in this memorandum).

e) It should also be noted that the labels for the 40 WP and 60 DF formulations still restrict use to fresh market apples and grapes only and prohibit the grazing of livestock in treated areas or the feeding of cover crops grown in treated areas to livestock. These restrictions were applicable to the earlier EUP program but are now inconsistent with the most recent requests for registrations and tolerances.

11. In the 40W formulation, there are two inerts for which the Agency has no information. The Registrant is requested to supply the necessary information needed to clear these inerts prior to registration. The identity of the inerts are given in a confidential appendix. The inerts in the 60 DF formulation have been cleared for this particular use.

12. Margin(s) of Safety (MOS) for Testicular Effects

In reviewing the submitted toxicity data, TB has identified a possible risk of testicular lesions following exposure to RH-3866 based upon results from a reproductive study in rats (see memorandum from Jane Harris to Henry Jacoby, dated 2/12/86) and upon results from a chronic feeding/ oncogenicity study in rats (review attached to this memorandum). In order to assess the potential hazard to farmworkers, a margin of safety for RH-3866 needs to be calculated. A margin of safety is calculated by relating a NOEL from a pertinent toxicity study to an estimate of human exposure. TB presently has sufficient toxicological information (i.e. NOELs from pertinent studies) for the purpose of calculating MOS(s) for testicular lesions. TB does not have at this time, however, estimates of human exposure that have been validated and/or confirmed by Exposure Assessment Branch (EAB). TB intends to meet with EAB and PM Team #21 as soon as possible to discuss this matter and decide how this human exposure information might be provided to TB as expeditiously as possible. Following receipt of this information from EAB, TB will calculate MOS(s) for testicular lesions in farmworkers and address this matter fully in a future memorandum.

13. Several requirements for particular toxicity studies which were requested by TB in previous actions are now inconsistent with and/or negated by the requirements and/or waivers made in this present memorandum. Therefore, the following study requirements are rescinded:

Technical Product

Dermal absorption study	(memorandum from P. Hurley to L. Rossi, dated 5/29/87)
Dermal sensitization study	(memorandum from P. Hurley to L. Rossi, dated 12/7/87)
21-Day dermal study	(memorandum from P. Hurley to L. Rossi, dated 12/7/87)

In addition to the previous point, it should be noted that the memorandum from P. Hurley to L. Rossi, dated 12/7/87 stated that the rat metabolism study was incomplete. Since that time, TB has received and reviewed a metabolism study that was in the Residue Chemistry Branch files on this fungicide. The information in that study now completes the data requirements for distribution and metabolism.

Studies Reviewed

.....Technical.....

Study	Results	Core Classification
90-day feeding - mouse	NOEL 300 ppm, LOEL 1000 ppm hepatocytic hypertrophy, other liver effects.	Guideline
Teratology - rabbit/range finding	Maternal, embryofetotoxicity NOEL's 100 mg/kg/day. Not a teratogen up to 215 mg/kg/day (HDT).	Supplementary
Teratology - rabbit	Not teratogenic up to 200 mg/kg/day (HDT). NOEL maternal tox. 20 mg/kg/day, NOEL feto-embryotoxicity 60 mg/kg/day.	Minimum
2-4 week feeding - dog/ range finding	NOEL 250 ppm, LOEL 1000 ppm. Decrease in bodyweights and food consumption.	Supplementary
One-year feeding - dog	NOEL 100 ppm, LOEL 400 ppm. Hepatocellular hypertrophy, supporting effects in organ weights and clinical chemistry.	Minimum
Rat chronic feeding/ oncogenicity	NOEL 50 ppm, LOEL 200 ppm (testicular atrophy). No oncogenic effects observed in doses up to 800 ppm.	Guideline for Chronic; Supplementary for Oncogenicity
Mouse Chronic feeding/ oncogenicity	NOEL 20 ppm, LOEL 100 ppm increase in liver mixed function oxidase. No oncogenic effects observed in doses up to 500 ppm.	Guideline for Chronic; Supplementary for Oncogenicity
In Vitro Cytogenetics	Does not induce chromosomal aberrations in doses up to limit of solubility.	Acceptable
Dominant Lethal - rat	Did not induce dominant lethal mutations in rats up to 735 mg/kg. Needs positive control data.	Unacceptable
Dermal Absorption - rats	Failure to perform analysis of application site skin and residue in carcass made it impossible to verify recovery.	Unacceptable

Study	Results	Core Classification
Metabolism - rat	Extensively metabolized and excreted in urine + feces. At least 7 major metabolites recovered + identified. Highest amounts of radioactivity found in liver, kidneys, large + small intestines.	Acceptable in combination with following rat metabolism study.
Metabolism - rat	Completely and rapidly absorbed. Extensively metabolized and rapidly and essentially completely excreted. Elimination of label from plasma biphasic and evenly distrib. between urine + feces. No tissue accumulation after 96 hours.	Acceptable in combination with previous metabolism study.
Metabolism - mice	Rapidly absorbed and excreted. Dose completely eliminated by 96 hours. Extensively metabolized.	Acceptable
	.....40 WP and 2EC	
4-week dermal - rat	Systemic NOEL for both formulations is 100 mg a.i./kg/day (HDT) and NOEL for skin irritation is 10 mg a.i./kg/day.	Minimum

8-Point Review

[Prepared for 707-ERE;ERN;ERR/7F3476; 7H5524, RH-3866 on apples, grapes, processed commodities, meat, milk and eggs, November 23, 1987]

1. Toxicity data with technical grade RH-3866 considered in support of these tolerances (selected studies).

Acute oral LD_{50}, rat	1.6 g/kg in males 2.3 g/kg in females
90-day feeding, rat	NOEL: 1000 ppm (50 mg/kg/day); LOEL: 3000 ppm (150 mg/kg/day)-increased liver, kidney wts.; hypertrophy, necrosis in liver; pigmentation in convoluted kidney tubules.
90-day feeding, dog	NOEL: 10 ppm (0.3 mg/kg/day); LOEL: 200 ppm (5.9 mg/kg/day)-hepatocellular hypertrophy.
One-year chronic feeding, dog	NOEL: 100 ppm (2.5 mg/kg/day); LOEL: 400 ppm (10 mg/kg/day)-hepatocellular hypertrophy, supporting evidence in organ weights and clinical chemistry.
Two-year chronic feeding/ oncogenicity, rat	NOEL: 50 ppm (2.5 mg/kg/day); LOEL: 200 ppm (10 mg/kg/day)-testicular atrophy. No oncogenic effects observed at doses up to and including 800 ppm (HDT). MTD may not have been reached.
Two-year chronic feeding/ oncogenicity, mouse	NOEL: 20 ppm (3.0 mg/kg/day); LOEL: 100 ppm (15 mg/kg/day)-increase in liver mixed function oxidase. No oncogenic effects observed at doses up to and including 500 ppm (HDT). MTD may not have been reached.
Teratology, rat	Not teratogenic up to and including 469 mg/kg/day (HDT). Maternal NOEL: 313 mg/kg/day. Developmental NOEL: 31 mg/kg/day-increased resorptions and decreased viability index.

Teratology, rabbit	Not teratogenic up to and including 200 mg/kg/day (HDT). Maternal NOEL: 20 mg/kg/day. Developmental NOEL: 60 mg/kg/day - increased number of resorptions and decreased viability index.
2-Generation reproduction, rat	Systemic NOEL: 50 ppm (4 mg/kg/day); LOEL: 200 ppm (16 mg/kg/day)- increased liver weights, hepatic hypertrophy in males. Reproductive NOEL: 200 ppm (16 mg/kg/day). LOEL: 1000 ppm (80 mg/kg/day)- testicular, epididymal and prostatic atrophy in P_2 males, incr. stillborns, decr. bdywt. gain in pups during lactation in F_1 and F_2.
Metabolism - rats	At least 7 major metabolites recovered and identified. Highest amounts of radioactivity found in liver, kidneys, large and small intestines. No tissue accumulation.
Metabolism - rats	Completely and rapidly absorbed. Extensively metabolized and rapidly and essentially completely excreted. Elimination of label from plasma biphasic and evenly distrib. between urine + feces. No tissue accumulation after 96 hours.

Mutagenicity Studies

Reverse mutation assay (Ames), point mutation in CHO HGPRT cells, in vitro and in vivo (mouse) cytogenetic assays, unscheduled DNA synthesis and dominant lethal study in rats. All the tests were negative and acceptable except Dominant Lethal (negative) which needs positive control data.

2. Additional toxicity data considered desireable: The MTD may not have been reached in the two rodent chronic feeding/oncogenicity studies. Further information or repeat studies are needed. Positive control data needed for Dominant Lethal study.

3. A request for the additional data (see above) is in the process of being sent to the Registrant (November, 1987).

4. A temporary tolerance of 0.5 ppm for the raw agricultural commodities, apples and grapes (fresh market only) has been issued. Applications for temporary tolerances for processed commodities of apples and grapes, and meat, milk and eggs have been filed by the Registrant. The Toxicology Branch (TB) had no objections to granting these temporary tolerances but TB has not been notified as to whether or not they have been issued.

5. The relationship of these tolerances on the contribution to the diet and the MPI must be addressed by the Residue Chemistry Branch and the TAS system.

6. The two-year chronic feeding study in rats with a safety factor of 100 was used to calculate the ADI. The NOEL was 50 ppm (2.5 mg/kg/day). The ADI is calculated to be 0.025 mg/kg/day and the MPI is calculated to be 1.5 mg/day (60 kg).

7. There are no pending regulatory actions against registration of the pesticide.

8. None.

MYCLOBUTANIL 128857

Page_____ is not included in this copy.

Pages 13 through 23 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

X A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

____ FIFRA registration data.

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

____ The document is not responsive to the request.

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.

eviewed by: Pamela Hurley
ection 2 , Tox. Branch (TS-769C)
econdary Reviewer: Edwin Budd
ection 2 , Tox. Branch (TS-769C)

Budd 11/11/88

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DATA EVALUATION REPORT

STUDY TYPE: Subchronic Feeding - mouse TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266079

TEST MATERIAL: RH-3866

SYNONYMS: Rally, Systhane, Myclobutanil

REPORT NUMBER: 83R-136

SPONSOR: Rohm & Haas Co., Philadelphia, PA

TESTING FACILITY: Rohm & Haas Toxicology Dept., Spring House, PA

TITLE OF REPORT: RH-3866: A Three-Month Dietary Toxicity Study in Mice

AUTHOR(S): P.R. Goldman, J.C. Harris, K.R. Lampe

REPORT ISSUED: October 8, 1986

IDENTIFYING VOLUME: Vol. 5 of 47

CONCLUSION: The NOEL for this study is 300 ppm based upon hepatocytic hypertrophy and other liver effects. The LOEL is 1000 ppm. The dose levels tested included levels of 3-10,000 ppm in the diet.

Classification: Guideline

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile
Description: brown-colored solid
Batch #(s), Other #(s): Sample No. 83-076, Lot No. LSPL0016 E
Purity: 81.1%
Source: Rohm & Haas

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Crl:CD®-1(ICR)BR mice, male and female
Age: 8 weeks
Weight(s): 32-34 g (males), 24-26 g (females)
Source(s): Charles River Breeding Labs, Stone Ridge, N.Y.

3. Procedure:

a. Dietary Preparation (if applicable): A jar of the technical sample is heated each week until liquid (50-60°C). The appropriate amount was weighed, dissolved in 50 ml acetone and mixed with feed in a hood to evaporate the acetone.

Frequency of preparation: weekly

Storage conditions: room temperature

Stability Analyses: Selected samples from the weekly preparations at each dose level were taken for stability analysis.

Homogeneity Analyses: Samples from the top, middle and bottom of each dietary concentration were collected the first time the diets were prepared and submitted for analysis of homogeneity of mixing.

Concentration Analyses: Conducted from the stability analysis studies.

b. Animal Assignment and Dose Levels:

Test Group	Dose Administered (ppm)	Main Study 13 weeks male	female
Contr.	0	10	10
1	3	10	10
2	10	10	10
3	30	10	10
4	100	10	10
5	300	10	10
6	1000	10	10
7	3000	10	10
8	10000	10	10

c. Clinical Observations and Mortality: Observed daily for signs of toxicity. Physical examinations conducted weekly.

d. Body Weight Determinations: weekly

e. Food and/or Water Consumption: weekly

f. Ophthalmological Examinations (if applicable): Not done

g. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals):
At the end of the dosing period (13 weeks)

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	x	Mean corpuscular HGB (MCH)
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB conc.(MCHC)
x	Leukocyte count (WBC)*	x	Mean corpuscular volume (MCV)
x	Erythrocyte count (RBC)*	x	Red cell morphology*
x	Platelet count*		
x	Total plasma protein (TP)		* Only determined for mice in the control group and the 2 highest dose groups (3000 and 10,000 ppm)
x	Leukocyte differential count*		

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X	Electrolytes:	X	Other:
x	Calcium*	x	Albumin*
	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorus*	x	Cholesterol*
	Potassium*	x	Globulins
	Sodium*	x	Glucose*
	Enzymes:	x	Total bilirubin*
x	Alkaline phosphatase	x	Total protein*
	Cholinesterase		Triglycerides
	Creatinine phosphokinase*	x	A/G ratio
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
x	Gamma glutamyl transferase (GGT)		

3) Hepatic Mixed Function Oxidase Assays:

Liver sections were taken from 4 animals/sex group in each of the six highest dose groups (30 to 10,000 ppm). The liver sections were analyzed for hepatic mixed function oxidase activity through the use of benzphetamine and aminopyrine N-demethylation assays.

4) Urinalysis: Not conducted

h. Gross Necropsy: After the 13 week treatment period, all surviving mice were necropsied and all organs, tissues and body cavities were examined and gross abnormalities were recorded.

i. Histopathology: The tissues marked below were saved from all animals that were necropsied. Microscopic examinations were conducted on all tissues saved from all animals in the control and the two highest dose groups (3000 and 10000 ppm). Target tissues were examined in the lower dose groups until a NOEL was reached.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

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

j. Statistical Analyses: Residual plots of the numerical parameters were examined for normality and homogeneity of variance across treatment groups. Analysis of variance was used for the same parameters; some group means were compared using Duncan's Multiple Range Test and other groups were compared using the Least Square Means Test.

B. RESULTS:

1. Dietary Preparation: Samples were taken for analysis from weeks 1 (including samples obtained for homogeneity testing), 2, 3, 4, 8, 12 and 13. No measurable residue of technical RH-3866 was found in most of the control samples; residues up to 9 ppm were found in 3 control samples (considered to be sample contamination). Recovery for 1-day aged samples averaged 94 +/15%. No explanation was given as to why the sample was allowed to age only 1 day. Average dose levels ranged from 90-119% of the theoretical values, with an overall average of 106%. The individual data indicate that there may have been some difficulties in mixing the test chemical into the diet. For example, at the theoretical dose level of

1000 ppm, the measured levels of chemical in the diet were 1450, 1600 and 690 ppm for samples taken from the bottom, middle and top of the mixer, respectively.

2. Clinical Observations and Mortality: One male mouse fed 1000 ppm RH-3866 died during the course of the study. The death was not considered to be treatment-related. The gross pathology report on this animal included a mottled liver, redness of the glandular stomach and duodenum, and red fluid in the abdominal cavity. The only clinical sign which appeared to be treatment-related was the notation of scant fecal droppings in the 10,000 ppm group throughout the dosing period.

3. Body Weight Determinations: At 10,000 ppm, the body weights of both male and female groups were statistically significantly lower than controls at all times except for females at week 7. Although body weight gains were not analyzed, graphical representation indicates that there was a highly significant decrease in the high dose animals when compared to controls during the first week. Thereafter the body weight gains appear to be similar to controls. At 13 weeks, the differences in body weights were approximately 19 and 8% for males and females respectively when compared to control groups. At 3000 ppm, the male body weights were also statistically significantly lower than controls at most time points. These decreases averaged around 7% lower. None of the animals in any of the other dose groups were affected.

4. Food and/or Water Consumption: The food consumption of male mice at the 10,000 ppm level was reduced during the first week. Food consumption of females at this dose level was reduced throughout the treatment period, and was statistically significantly lower during the first week. Differences at other times were considered to be incidental.

5. Hematology: At 10,000 ppm, a significant decrease in the hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) values were observed in both sexes and an increase in mean corpuscular hemoglobin concentration (MCHC) was observed in both sexes. At this dose level, males also had a significant decrease in WBC and the number of lymphocytes and an increase in the number of segmented platelet values, and females had decreased hemoglobin and increased platelet values. For various reasons, none of the other observed changes were considered to be related to treatment.

6. Clinical Chemistry: At 10,000 ppm, increases in SGOT, SGPT, ALK, GGT and BUN were observed in both sexes. Increases in SGPT were also observed in both sexes at 3000 ppm and in males at 1000 ppm, although not statistically significant in females at 3000 ppm or in males at 1000 ppm. SGOT was increased in males at 3000 ppm, although not significantly so. Glucose levels were significantly reduced in both sexes at 10,000 ppm and in females at 3000 ppm. Cholesterol

values were decreased in both sexes at 10,000 ppm and 3000 ppm and in males at 1000 ppm. For various reasons, none of the other changes observed were considered to be related to treatment.

7. Gross Pathology: At 10,000 ppm and at 3000 ppm enlarged livers with accentuated lobular architecture were observed in both sexes. At 10,000 ppm, 18/20 animals were affected (20/20 with accentuated lobular architecture), and at 3000 ppm, 4/20 animals were affected. Other abnormalities observed were not considered to be treatment-related.

8. Organ Weights: Liver weights (absolute and relative) were increased in both sexes at the 1000, 3000 and 10,000 ppm dose levels. All increases were statistically significant except the absolute liver weights in 1000 ppm female mice. Absolute kidney weights were decreased in male mice at 10,000 ppm. No other changes in organ weights at any dose level were considered to be treatment-related. Some differences were considered to be probably due to decreased terminal body weights in some animals.

9. Hepatic Mixed Function Oxidase Assay: RH-3866 increased MFO activity on a per g liver basis (BP N-demethylation) in females at 300, 3000 and 10,000 ppm but had no effect at 1000 ppm. Therefore, the increase at 300 ppm was not considered to be biologically significant. MFO activity was increased in males at levels of 1000 ppm and greater.

10. Histopathology:

a. Nonneoplastic lesions: Microscopic changes in the liver were seen in males at dose levels of 1000 ppm and above and in females at dose levels of 3000 ppm and above. These observations included centrilobular or centrilobular and midzonal hepatocytic hypertrophy, swollen-vacuolated centrilobular hepatocytes, single large hepatocytic vacuoles, centrilobular individual cell hepatocytic necrosis and centrilobular necrotic hepatitis. Pigment in Kupffer cells was evident in both sexes at 3000 and 10,000 ppm. Bile duct proliferation was seen in both sexes at 10,000 ppm. The NOEL for liver effects. was 300 ppm in males and 1000 ppm in females. The microscopic findings for liver are summarized in table 1.

Other observed changes that were considered to be compound-related were cytoplasmic ecsinophilia and/or hypertrophy of the zona fasculata cells of the adrenal glands at 1000 ppm in males and at 3000 ppm and above in both sexes; the presence of pigment in the macrophages in the spleen (3000 ppm and above) and kidney (10,000 ppm); lymphoid necrosis in the spleen (2 males at 3000 ppm, 1 female at 10,000 ppm and 3 of each sex at 30,000 ppm); an increase in the myeloid:erythroid ratio, primarily involving the granulocytes in the bone marrow in some females at 10,000 ppm; immaturity of the uterus and absence of corpora lutea in the ovaries of females at

10,000 ppm and increased mononuclear cell infiltration in the skin in both sexes at 10,000 ppm. All other changes were considered to be spontaneous in nature and unrelated to treatment.

b. Neoplastic lesions: None

11. Quality Assurance Measures: The report and original data were reviewed by the Quality Assurance Unit of Rohm and Haas. To the best of their knowledge, the study did not deviate from the published GLP's. The report was signed.

C. DISCUSSION: The major compound-related effect observed in this study is hepatocytic hypertrophy, along with other liver effects. These effects were evident both on a gross level and on a microscopic level. In addition, they were supported by clinical chemistry evidence and by the organ weight data. There were several questions concerning the design and conduct of the study and there were slight deviations from the EPA Testing Guidelines, but these were not considered to be significant enough to affect the outcome of the study. There appeared to be a possible problem with homogeneity of mixing. This is pointed out in the results section. In addition, the stability analysis study was done for a sample which was only stored for one day. Since the diets were formulated weekly, the sample tested was not stored long enough prior to testing. No explanation was given as to why the test sample was not stored for at least a week. The Guidelines called for an ophthalmological examination. This was not done. The Guidelines also called for microscopic examinations on the lungs, livers and kidneys of all test groups. Only the liver and other target organs were examined microscopically in the other test groups besides the 2 top dose levels. Since all organs were examined in the 2 top test groups and since target organs were examined in the lower dose levels (at least until a NOEL was reached), this deviation from the Guidelines is not considered to be significant. This study is classified as CORE GUIDELINE.

Incidence of Nonneoplastic Microscopic Findings in the Liver

Dose Level (ppm)	0		3		10		30		100		300		1000		3000		10,000	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
# Livers Examined	10	10	0	0	0	0	0	0	10	10	10	10	10	10	10	10	10	10
Centrilobular Hepatocytic Hypertrophy	6	0	0	0	0	0	0	0	4	0	5	0	10	0	10	10	0	0*
Microgranuloma (5) Lymphoreticular Cell Infilt.	10	9	0	0	0	0	0	0	2	7	5	9	5	5	5	5	0	1
Swollen-Vacuolated Centrilobular Hepatocytes	0	0	0	0	0	0	0	0	0	0	0	0	3	0	10	5	10	10
Single Large Vacuoles, Hepatocytes	0	0	0	0	0	0	0	0	0	0	0	0	3	0	6	8	5	4
Individual Hepatocytic Necrosis, Centrilobular	0	0	0	0	0	0	0	0	0	0	0	0	3	0	8	2	0	0
Coagulative Necrosis	1	2	0	0	0	0	0	0	0	2	0	3	2	1	6	3	1	1
Pigment, Kupffer's Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	10	9
Foci of Granulopoeisis	1	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0	0	0
Congestion	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Periportal Granulomatous Inflammation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1
Centrilobular/Midzonal Hepatocytic Hypertrophy	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	10*
Necrotic Hepatitis, Centrilobular	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	2	10	10
Bile Ductule Proliferation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	7

* High dose animals had both centrilobular and midzonal hepatocytic hypertrophy

eviewed by: Pamela Hurley
ection 2 , Tox. Branch (TS-769C)
econdary Reviewer: Edwin Budd
ection 2 , Tox. Branch (TS-769C)

Budd 1/11/88

006580

DATA EVALUATION REPORT

STUDY TYPE: Chronic/Oncogenicity - Mouse (83-5) TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266090

TEST MATERIAL: RH-3866

SYNONYMS: Rally, Systhane, Myclobutanil

REPORT NUMBER: 84R-023

SPONSOR: Rohm & Haas Company, Philadelphia, PA

TESTING FACILITY: Toxicology Dept., Rohm & Haas Company, Spring House, PA

TITLE OF REPORT: RH-3866: Dietary Chronic and Oncogenicity Study in Mice

AUTHOR(S): P.R. Goldman and J.C. Harris

REPORT ISSUED: October 17, 1986

IDENTIFYING VOLUME: Volume 16 of 47

CONCLUSION: The NOEL was 20 ppm and the LOEL was 100 ppm (slight increase in liver mixed function oxidase). Microscopic changes in the liver were evident in both sexes at 500 ppm.

Classification: CORE GUIDELINE for chronic effects and CORE SUPPLEMENTARY for oncogenicity (see discussion).

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-4-chlorophenyl-1H-1,2,4-triazole-1-propanenitrile
Description: red-brown solid
Batch #(s), Other #(s): Sample # 83-260, lot # LAP-0298
Purity: 90.4%
Source: Rohm & Haas

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female Crl:CD®-1(ICR)BR mice
Age: 3 weeks upon receipt
Source(s): Charles River Breeding Labs

3. Procedure:

a. Dietary Preparation (if applicable): Sample was heated to approximately 70°C until liquified. Liquid was stirred to ensure homogeneity, weighed, dissolved in acetone and mixed with feed in a hood to evaporate the acetone.
Frequency of preparation: weekly
Storage conditions: at room temperature, in dark dry area
Stability Analyses: Each week, an extra feed cup for each dietary level was prepared and left on top of the cage rack in the study room for the treatment week, collected and then submitted for analysis in order to verify stability.
Homogeneity Analyses: The first time the diet was prepared, samples from the top, middle and bottom were taken for analysis.
Concentration Analyses: all samples obtained to assess adequacy of mixing and those obtained during first month for quality assurance were analyzed as well as one sample from each dietary concentration per month. Other samples were preserved and sent to analysis group.

b. Basis For Selection of Dosage Levels:

Not stated, but probably based upon results of subchronic study that was conducted.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 24 months		Interim Sac. 3 months		Interim Sac. 6 months		Interim Sac. 12 months	
		male	female	male	female	male	female	male	female
Contr.	0	70	70	10	10	10	10	20	20
1	20	70	70	10	10	10	10	20	20
2	100	70	70	10	10	10	10	20	20
3	500	70	70	10	10	10	10	20	20
4	Sentinel	25	25	-	-	-	-	-	-

d. Clinical Observations and Mortality: Animals observed daily for signs of ill health and reaction to treatment. Physical exams conducted weekly for first 14 weeks and at 2 week intervals thereafter.

e. Body Weight Determinations: weekly

f. Food and/or Water Consumption: weekly

g. Ophthalmological Examinations (if applicable): 12 and 24 months

h. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals):
3, 6, 12 and 24 months; 10/sex/group at 3 and 6 months, 15/sex/group at 12 and 24 months.

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	x	Mean corpuscular HGB (MCH)
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB conc.(MCHC)
x	Leukocyte count (WBC)*	x	Mean corpuscular volume (MCV)
x	Erythrocyte count (RBC)*	x	Red cell morphology†
x	Platelet count*		
	Total plasma protein (TP)		
x	Leukocyte differential count*†		†Only on high dose and controls

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X		X	
	Electrolytes:		Other:
x	Calcium*	x	Albumin*
	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorus*	x	Cholesterol*
	Potassium*	x	Globulins
	Sodium*	x	Glucose*
	Enzymes:	x	Total bilirubin*
x	Alkaline phosphatase	x	Total protein*
	Cholinesterase	x	Triglycerides
	Creatinine phosphokinase*	x	A/G ratio
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
x	Gamma glutamyl transpeptidase		

3) Urinalysis:

Collection times for urine (including # of animals): 6, 12 and 24 months from same animals as blood.

The following CHECKED (X) parameters were examined:

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

4) Hepatic Mixed Function Oxidase (MFO) and Peroxisomal Beta-Oxidation Analyses

At 3, 6, and 12 months, livers from 6 mice/sex/group were randomly selected from animals scheduled for post-mortem examinations and analyzed for MFO activity. Additional samples taken from the 12 month sacrifice were frozen and subsequently analyzed for hepatic peroxisomal beta-oxidation activity.

i. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations:

All animals.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations:

All animals.

j. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination:

Tissues were preserved from all animals. Only liver was examined microscopically from animals scheduled for sacrifice at 3 and 6 months. At 12 months, all tissues listed below examined for controls and high dose; liver, gross lesions and tissue masses were examined for other dose groups. All tissues examined for non-surviving mice in all dose groups.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination:

At 24 months, all tissues examined in controls and high dose groups; liver, kidneys, lungs, testes and tissues with gross changes were examined in other dose groups. Tissues examined from sentinel mice were brain, liver, kidneys, lung, spleen, liver, colon and other tissues with gross changes.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

X		X		X	
	Digestive system		**Cardiovasc./Hemat.**		**Neurologic**
	Tongue		Aorta*	xx	Brain*
x	Salivary glands*	xx	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	xx	Spleen*	x	Eyes (optic n.)*
x	Jejunum*	x	Thymus*		**Glandular**
x	Ileum*		**Urogenital**	xx	Adrenals*
x	Cecum*	xx	Kidneys*		Lacrimal gland
x	Colon*	x	Urinary bladder*	x	Mammary gland*
x	Rectum*	xx	Testes*	x	Parathyroids*
xx	Liver*	x	Epididymides	x	Thyroids*
x	Gall bladder*	x	Prostate		**Other**
x	Pancreas*	x	Seminal vesicle	x	Bone*
	Respiratory	xx	Ovaries	x	Skeletal muscle*
x	Trachea*	x	Uterus*	x	Skin
x	Lung*	x	Vaginat	x	All gross lesions and masses
x	Larynx	x	Coagulating gland†		

† Saved at 12 and 24 months only. The coagulating gland/seminal vesicles/prostate glands were collected at necropsy as a single unit.

k. Statistical Analyses: Body weights, feed consumption, clinical chemistry, hematology, urinalysis and organ weights inspected for normality and homogeneity of variance across treatment groups by examining residual plots. Analysis of variance used in assessments for overall treatment effect; group means compared using Duncan's test when significant treatment effect found. Survival distributions compared separately within each sex and also pooled over sex by using both logrank and Wilcoxon tests found in PROC LIFETEST of the Statistical Analysis System (SAS).

B. RESULTS:

1. Dietary Preparation: Samples for week 1 homogeneity analyses as well as samples retained from weeks 1, 2, 4 and those taken from 4 week intervals were analyzed for RH-3866 concentration. The overall average concentrations for each dose level ranged from 92-105% of the theoretical dosages. The average for the 3 concentrations together was 98%. The individual concentrations for each dose level ranged from 11-40 ppm for the 20 ppm dose level, from 57-130 ppm for the 100 ppm dose level and from 280-890 ppm for the 500 ppm dose level. Obviously, the extreme deviations from the theoretical dose levels did not occur very often.

2. Clinical Observations and Mortality: There was no apparent effect of the test chemical on the survival of the test groups. Percent survival at the end of the 24 month treatment period was 50, 47, 44, and 56% for the controls, 20, 100, and 500 ppm groups, respectively for males and 46, 51, 47 and 51% for the females, respectively. No treatment-related signs of clinical toxicity were observed in any of the test groups. The following signs were observed in all groups: red swollen ears, alopecia, arched back and yellow stained anogenital area. According to the authors, some mice showed some common pre-death signs associated with a debilitated state. These signs included ataxia, tremors and lethargy.

3. Body Weight Determinations: No treatment related changes in body weight were observed in any of the test groups. Significantly decreased body weights were observed when compared to controls at individual times in the highest dose group (only once in the mid-dose group in females after the pretest period), but these were not consistent.

4. Food and/or Water Consumption: No dose-related changes in food consumption were observed with any of the treated groups. Sporatic statistically significant increases and decreases in food consumption were observed in all of the treated groups.

5. Ophthalmological Examinations: No treatment-related abnormalities were observed in any of the treated groups.

6. Hematology: No treatment-related changes were observed in any test group. A significant increase in the mean corpuscular hemoglobin concentration value was observed in female rats at the 20 ppm dose level at 6 months. This did not occur in any of the higher dose levels, nor did it occur again at any of the other time periods. Therefore, it was considered to be spurious.

7. Clinical Chemistry: After 3 months of treatment, SGPT values were increased in female mice at 500 ppm. This was considered to be a treatment-related effect since an increase in MFO activity and an increase in liver weights were observed at this time. Other changes observed at this time were considered to be questionable in terms of their biological significance because they were not seen at later time periods and they were not observed with higher dose levels that were tested in a previous study (mouse subchronic feeding). No treatment-related changes were observed in any of the treated groups at any of the other time periods.

8. Urinalysis: No treatment-related effects were observed in any of the test groups.

9. Hepatic Mixed Function Oxidase Assay: After 3 months of treatment with the test chemical, MFO activity was increased in females at 100 ppm and in both sexes at 500 ppm. At this time point, RH-3866 had no effect on hepatic microsomal protein content at any dose level. After 6 months of treatment, MFO activity was increased in both sexes at 500 ppm. At this dose level, hepatic microsomal protein content was increased 28% and 21% in males and females, respectively. After 12 months of treatment, significant increases in MFO activity were observed in 500 ppm females. Hepatic microsomal protein concentration was not affected at any dose level after 12 months. At this time period, RH-3866 had no effect on peroxisomal ^{14}-palmitoyl-CoA oxidase activity.

10. Gross Pathology: No treatment-related gross changes were observed in any of the treated groups at any time period.

11. Organ Weights: At 3 months, absolute and relative liver weights were significantly increased over controls in both male and female mice fed 500 ppm of the test chemical. No treatment-related changes in organ weights were observed at any dose level at either 6 months, 12 months or 24 months. All the changes that were observed were considered to be either spurious or due to the fact that the control weights were exceptionally low.

12. Histopathology:

    a. Nonneoplastic lesions: Treatment-related changes in the liver were observed in male mice at the 500 ppm level after 3, 6, and 12 months of treatment. These changes included increased incidences and severity of centrilobular hepatocytic hypertrophy,

Kupffer cell pigmentation, periportal punctate vacuolation and individual hepatocellular necrosis. Following 24 months of treatment, changes in the liver were observed in both sexes at the 500 ppm level. These included increased incidences of focal hepatocellular alterations and multifocal hepatocellular vacuolation. These were not associated with any hypertrophy, hyperplasia or neoplastic proliferations in the liver. No other treatment-related microscopic changes were observed at any dose level. Table I summarizes the liver effects observed.

b. Neoplastic lesions: No treatment-related increases in any neoplasms were observed at any dose level. Hepatocellular hypertrophy and hepatocellular adenomas or carcinomas occurred in mice of all groups, including the controls. RH-3866 had no effect on the severity or incidence of these lesions. Other neoplastic lesions of the liver were also found in all groups: hemangioma, hemangiosarcoma, lymphoreticular lesions, and metastatic or invasive tumors. Adenomas and carcinomas of the lungs were found in all groups as well as lymphosarcomas and other neoplastic lesions of the lymphoreticular system. The attached tables summarize the incidence of neoplastic lesions found. Two tables are given, one for mice which either died or were sacrificed prior to and including 12 months and one for mice which either died or were sacrificed at the termination of the study.

c. Sentinel mice: There was no indication of any intercurrent disease.

13. Quality Assurance Measures: The study was audited and reviewed numerous times by the Quality Assurance Unit for adherence to GLP's and the final report was signed by this group.

C. DISCUSSION: As a chronic feeding study, this appears to be a well conducted study. It is classified as CORE GUIDELINE for a chronic feeding study. The study is classified CORE SUPPLEMENTARY as an oncogenicity study because the Toxicology Branch (TB) does not believe that the top dose level tested was sufficiently high enough. It does not appear that the Maximum Tolerated Dose (MTD) was reached. The effects seen at the highest dose level tested were increases in liver mixed-function oxidase, SGPT and liver weights; and microscopic alterations of the liver consisting of centrilobular hypertrophy, vacuolation, Kupffer cell pigmentation and altered foci (eosinophilic, basophilic, vacuolated and clear cell types). These effects are not considered to be sufficiently severe enough to establish that the highest level tested (500 ppm) approached the MTD.

This chemical was tested in a 3-month dietary subchronic study. The dose levels tested were approximately 3, 10, 30, 100, 300, 1000, 3000 and 10,000 ppm. No effects were observed with dose levels up to and including 300 ppm. At 1000 ppm, increased liver enzyme activity and

liver weights were observed as well as hepatocytic hypertrophy, vacuolation and a borderline count of individual hepatocytic necrosis. At 3000 ppm, in addition to the effects noted above, an increase in SGOT in males (although not statistically significant) and pigmentation of the Kupffer cells were observed. A more significant count of individual hepatocytic necrosis was seen but this was not noted in any of the animals exposed to 10,000 ppm. At 10,000 ppm, increases in liver enzymes, BUN, and kidney weights were noted in addition to the effects observed above as well as some hematological effects. Clearly, at this dose level more significant toxicological effects were being observed. Based upon the effects noted in the subchronic study in mice, TB believes that higher dose levels should have been used in the mouse chronic study.

Summary of Nonneoplastic Liver Effects Observed in Mice After Chronic Exposure to RH-3866

Observed Effect	Dose Level							
	Males				Females			
	0 ppm	20 ppm	100 ppm	500 ppm	0 ppm	20 ppm	100 ppm	500 ppm
Hepatocellular Centrilobular Hypertrophy								
3 months	1/10	1/10	1/10	9/10				
6 months	2/10	2/10	1/10	9/10				
12 months	5/20	6/20	5/20	16/20				
12-24 months	8/66	6/63	5/65	11/62				
Sentinel (12 mo.)	1/5							
Kupffer Cell Pigmentation								
6 months	0/10	0/10	0/10	5/10				
12 months	4/20	1/20	4/20	12/20				
Sentinel (12 mo.)	2/5							
Periportal Punctate Vacuolation								
3 months	0/10	0/10	0/10	2/10	0/10	0/10	0/10	1/10
6 months	0/10	0/10	0/10	3/10	0/10	0/10	1/10	2/10
12 months (multifocal)	0/20	0/20	0/20	4/20	0/20	1/20	1/20	3/20
Sentinel (multifocal)	0/5	1/5						
Individual Hepatocellular Necrosis								
6 months	1/10	1/10	3/10	3/10				
12 months	2/20	1/20	1/20	6/20	0/20	0/20	1/20	2/20
Sentinel (12 mo.)	2/5	0/5						

Summary of Liver Effects (Continued)

Observed Effect	Dose Level							
	Males				Females			
	0 ppm	20 ppm	100 ppm	500 ppm	0 ppm	20 ppm	100 ppm	500 ppm
Focal Hepatocellular Alterations								
focus/foci, basophilic	2/66	3/63	1/65	4/62	0/64	0/66	1/66	2/67
focus/foci, clear-cell	0/66	0/63	0/65	2/62	0/64	0/66	0/66	0/67
focus/foci, eosinophilic	2/66	1/63	4/65	5/62	2/64	2/66	1/66	4/67
focus/foci, vacuolated cell	0/66	0/63	1/65	0/62	0/64	0/66	0/66	0/67
Total incidence	4/66	4/63	5/6/65*	10/11/62*	2/64	2/66	2/66	6/67
Multifocal Hepatocellular Vacuolation								
centrilobular	1/66	1/63	0/65	1/62	0/64	1/66	0/66	0/67
diffuse	0/66	0/63	0/65	0/62	0/64	0/66	0/66	1/67
multifocal	1/66	0/63	2/65	7/62	3/64	2/66	0/66	7/67

* / = number of mice with hepatocellular alteration/actual incidence of hepatocellular alteration. In 4 instances, a mouse had more than 1 type of hepatocellular alteration (or neoplasia, which were not included in this table).

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Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

Budd 11/11/88

006580

DATA EVALUATION REPORT

STUDY TYPE: Rat Chronic/Oncogenicity (83-5) TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266081

TEST MATERIAL: RH-3866

SYNONYMS: Myclobutanil, Systhane, Rally

STUDY NUMBER(S): Sponsor's Project No. 85RC-61, Testing Lab Project No. 8342

SPONSOR: Rohm and Haas Company, Spring House, PA

TESTING FACILITY: Tegeris Laboratories, Inc. Laurel, MD

TITLE OF REPORT: Chronic Toxicity and Oncogenicity Study with RH 3866 in Rats

AUTHOR(S): T.E. Shellenberger, L.H. Billups, A.S. Tegeris, D.S. Green

REPORT ISSUED: 10/24/86

IDENTIFYING VOLUME: Volumes 7-13 of 47

CONCLUSION: The NOEL for the study is 2.49 mg/kg/day and the LOEL is 9.84 mg/kg/day based upon testicular atrophy in males. No other significant effects were observed in either sex. The overall mean daily consumption was 0, 2.49, 9.84 and 39.21 mg/kg/day for males and 0, 3.23, 12.86 and 52.34 mg/kg/day for females for the controls, low, mid- and high dose groups, respectively. No oncogenic effects were observed.

Classification: CORE GUIDELINE for the chronic portion of the study and CORE SUPPLEMENTARY for the oncogenicity portion of the study (see discussion).

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-4-chlorophenyl-1-H-1,2,4-triazole-propanenitrile
Description: Solid or viscous solid
Batch #(s), Other #(s): TD #83-260, Lot # LAP 0298 (first 15 weeks); TD #84-038, Lot # 83159-7 (weeks 16ff)
Purity: 90.4% a.i.: 91.4% a.i. (due to error in initial labeling from Sponsor, the dietary conc. of second batch calculated from a value of 92.7% a.i.)
Source: Rohm & Haas

51

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female Sprague-Dawley rats
Age: 6-8 weeks, dosing to begin after 2-3 week acclimatization period.
Weight(s): Approx. 130-140 grams (F-M, 1 week prior to initiation of study)
Source(s): Charles River Breeding Laboratories, Wilmington, MA

3. Procedure:

a. Dietary Preparation (if applicable): Each jar was heated in a water bath until the sample was liquified; the temperature of water bath did not exceed 90°C. The liquified test chemical was stirred and small aliquots were placed in jars until ready to be used. When used, the sample was heated again, weighed, dissolved in acetone and mixed in the feed. The acetone was evaporated off.
Frequency of preparation: weekly
Storage conditions: room temperature
Stability Analyses: 2-week stability test at all dose levels prior to study
Homogeneity Analyses: Pretest analysis. Samples from top, middle and bottom portions of the feed mixer were retained for analyses. Samples were stored in animal room in feeders and samples obtained after 1 and 2 weeks for assay of compound concentration.
Concentration Analyses: Samples collected at each dose level throughout the study and frozen. Analyses for dose level verification were conducted weekly during first four weeks and subsequently from one set of samples every 4 weeks during the remainder of the study.

b. Basis For Selection of Dosage Levels:

Not stated, however, a subchronic feeding study in rats has been conducted in which the NOEL was 1000 ppm and the LEL was 3000 ppm based upon liver and kidney effects.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered (ppm) Weeks 1-2	Weeks 3-4	Weeks 5 to term
1-Control	0	0	0
2	25	35	50
3	100	140	200
4	400	560	800

Number of Animals Sacrificed:

Test Group	Main Study 24 months male	female	Interim Sac. 3 months male	female	Interim Sac. 6 months male	female	Interim Sac. 12 months male	female	Interim Sac. 17 months male	female
1	all surviving		10	10	10	10	20	20	18	10
2	all surviving		10	10	10	10	20	20	18	10
3	all surviving		10	10	10	10	20	20	18	10
4	all surviving		10	10	10	10	20	20	18	10

A sentinel animal program was also used with this study. Thirty male and 30 female rats were used. Prior to the initiation of the study, 5 animals of each sex were subjected to a complete viral and microbiological evaluation. At 3, 6 and 12 months, blood sera and/or live animals were submitted to the diagnostic laboratory for evaluation.

d. Procedures for Studies Other Than Feeding and/or Additions, Changes in Feeding Study: Dietary levels were adjusted on the basis of active ingredient content of the test material. Dietary levels were also adjusted during the initial 5 weeks of the study in order to provide a more nearly equal compound intake, mg/kg/day, during the active growth period of the animals.

e. Clinical Observations and Mortality: Twice daily. Detailed examinations when body weights were measured.

f. Body Weight Determinations: -1 weeks, 0, weekly during first 14 weeks, 1x every two weeks thereafter.

g. Food and/or Water Consumption: -1 weeks, weekly during first 14 weeks, 1x every two weeks thereafter.

h. Ophthalmological Examinations (if applicable): Prior to 12-month and terminal necropsies. Performed on all controls and high dose animals. Will be conducted on other animals if effects noted in high dose animals.

i. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals):
10 males, 10 females/group at 3, 6, 12, 17 months and prior to termination of study.

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	x	Mean corpustular HGB (MCH)
x	Hemoglobin (HGB)*	x	Mean corpustular HGB conc.(MCHC)
x	Leukocyte count (WBC)*	x	Mean corpustular volume (MCV)
x	Erythrocyte count (RBC)*	x	Red cell morphology#
x	Platelet count*		
	Total plasma protein (TP)		
x	Leukocyte differential count*#		

Evaluated only on control and high-dose groups at 6 and 12 months and in all dose groups at 3 months.

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X		X	
	Electrolytes:		Other:
x	Calcium*	x	Albumin*
	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorus*	x	Cholesterol*
	Potassium*	x	Globulins
	Sodium*	x	Glucose*
	Enzymes:	x	Total bilirubin*
x	Alkaline phosphatase	x	Total protein*
	Cholinesterase	x	Triglycerides#
	Creatinine phosphokinase*	x	A/G ratio
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
x	Gamma glutamyl transpeptidase (GGTP)		

12-month and terminal sacrifice only

3) Urinalysis:

Collection times for urine (including # of animals):
Control and high-dose groups designated for hematology and clinical chemistry collected at 3 (all dose groups), 5, 11, 17 months.

The following CHECKED (X) parameters were examined:

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

j. Liver Enzyme Assays: Sections of liver from 6 males and 6 females in the control, low, mid and high-dose groups were obtained at the 3, 6 and 12-month sacrifices for determination of mixed function oxidase (MFO) activity. MFO activity was measured by the in vitro enzyme assay of demethylation of aminopyrine (AP). At the 12-month necropsy, livers from 5-6 males and females randomly selected from each group were collected and analyzed for peroxisomal beta-oxidation activity utilizing ^{14}C-palmitoyl-CoA as substrate.

k. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations:

10/sex/group at 3 and 6 months; 20/sex/group at 12 months; 18 males and 10 females at 17 months. All animals found dead or sacrificed in a moribund condition.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations:

All animals.

l. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination:

All animals for liver, testes and ovaries; lungs and kidney: all animals at 12 and 17 months; all organs at 12 months in control and high-dose groups and target organs in mid- and low-dose groups; all organs in animals that were found dead and sacrificed moribund; gross lesions and masses: control and high-dose males and females at 3, 6 and 17 months, all animals at 12 months, all animals that died or were sacrificed moribund.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination:

Liver, testes, ovaries, lungs, kidneys, gross lesions and masses in all animals. Otherwise, all tissues required by protocol in control and high-dose groups and target organs in mid and low-dose groups. In addition, other tissues not required by protocol occasionally were inadvertently examined in some males and females at the 12 and 17-month interim and terminal sacrifices.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

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

m. Statistical Analyses: one-way Analysis of Variance followed by Dunnett's t-test. Percent survival estimated with Lifetest Procedure (SAS Institute).

B. RESULTS:

1. Dietary Preparation: Measured dose levels ranged between 83-108% of the desired levels. The average was 95%.

2. Clinical Observations and Mortality: Treatment with the test chemical did not affect the survival of males or females at any dose level. No difference in mortality was noted. By week 105 the total mortalities in males were 35, 35, 32 and 30 in the control, low, mid and high-dose groups, respectively, and 37, 39, 40, and 35 in females respectively. There were no clinical signs observed

that appeared to be related to treatment. The most common clinical signs that were observed throughout the study in all groups, including controls were dermal alopecia, rough haircoat, rash, footpad swelling and mechanical injuries.

3. Body Weight Determinations: The mean weekly body weights of the treated males were similar to controls during the first 8 weeks of the study. After that time, the mean weekly body weights of the high dose males began to decline relative to the control values and by week 22, they were significantly less than controls. This continued up to week 40. Although the body weights of the high dose males were statistically significantly less than the controls, the values still remained within 95-97% of the control values. After that time, the mean body weights of the high dose males were always less than controls, but were only significantly less at weeks 56, 80, 82 and 84. The data suggest that the test chemical induced a decrease in the mean body weights of the males in the high dose group when compared to controls between 6 and 18 months. The body weights at the lower dose levels were generally slightly lower than controls during this time period, but the lower values were not considered to be biologically significant. For females, the test chemical appeared to have no effect on the body weights of the treated animals during the first year of the study. During the second year, the test chemical appeared to have a marginal effect on the body weights of the high dose females relative to controls. The body weights were generally lower than controls during weeks 54 to 96 and the differences were statistically significant at weeks 66-72, 76-84 and 92. During weeks 76-84, the body weights were generally between 88-90% of the control values.

4. Food and/or [illegible] Consumption: For males, food consumption was generally lo[illegible] in the high dose animals when compared to controls, starting around the fifth week. Food consumption was statistically significantly lower for fifteen weeks within the fifth to seventy-eighth week; however, the values never dropped below 91% of the control values. Beginning at week 80, food consumption was similar to controls. No significant differences were noted for the two lower dose male groups. For females, in general, no significant changes in food consumption were observed between the treated and control animals. Based upon the food consumption values, the overall mean daily compound consumption for males was 0, 2.49, 9.84 and 39.21 mg/kg/day and for females was 0, 3.23, 12.86 and 52.34 mg/kg/day for controls, low, mid- and high dose groups, respectively.

5. Ophthalmological Examinations: There were no indications of compound-related ocular abnormalities. The most prevalent abnormality observed prior to termination of the study was diagnosed as conjunctivitis secondary to infectious diseases or dental abnormality.

6. Hematology: No treatment-related differences were observed in any of the treated groups when compared to controls.

7. Clinical Chemistry: There were a number of occurrences of statistically significant differences in several clinical chemistry values. However, for various reasons, each one of these were considered to be biologically insignificant. Therefore, there were no treatment-related changes observed in any of the clinical chemistry parameters.

8. Urinalysis: No consistent differences were noted between the treated and control groups.

9. Hepatic Enzyme Assays: A slight increase in hepatic mixed function oxidase (MFO) activity (34-47%) was seen in high-dose level males at 3, 6 and 12 months. The increases were statistically significant at 3 and 6 months. MFO activity was significantly increased in females in both mid- and high dose groups (61% and 78%, respectively) at 3 months. At 6 and 12 months, slight increases in high dose females were observed but were not statistically significant. RH-3866 had no effect on hepatic peroxisomal ^{14}C-palmitoyl-CoA oxidase activity in rats at dose levels up to 800 ppm after 12 months of dietary treatment.

10. Gross Pathology: The distributions and incidences of palpable masses in both males and females, as well as the mean "time-to-tumor" for the masses indicate that the presence of the masses were unrelated to treatment. The number of animals in each dose group with palpable masses was 1, 7, 2 and 4 in males for the first 12 months; 15, 13, 16 and 9 in males for months 13 to termination; 7, 9, 13 and 2 in females for the first 12 months; and 47, 46, 38 and 39 in females for months 13 to termination in control, low, mid- and high-dose groups respectively.

    All gross lesions observed at 3 and 6 months were considered to be unrelated to treatment. This was also true for the gross lesions observed at 12 months except for testicular lesions. Testicular reduction in size was seen in 0, 1, 1 and 3 animals in the control, low, mid and high dose animals, respectively. The higher incidence observed in the high dose animals correlates with the reduction in testicular weights in this dose group. At 17 months, again, only the testicular effects were considered to be related to treatment with the test chemical. Bilateral reduction in testicular size was observed in 2, 2, 0 and 6 animals in the control, low, mid and high dose groups, respectively. At terminal sacrifice, with the exception of the testicular effects, all observed lesions appeared to be unrelated to treatment. Decrease in testicular size was seen in 0, 2, 7 and 6 males respectively, in controls, low, mid- and high dose animals. In addition, a second testicular lesion characterized as "soft" was also seen in 1, 1, 5, and 5 males in the controls, low, mid- and high dose groups, respectively. Reduction in testicular size was also seen in the treated animals that died prior to the completion of the study (2, 6, 2 and 7 in controls, low, mid- and high dose animals, respectively).

11. Organ Weights: In males, no significant differences were observed between mean liver weights and between mean liver-to-body weight ratios in the treated animals versus the controls at any of the sacrifice times. In females, the mean liver-to body-weight ratio in the high dose animals was significantly higher than controls at 3 months (113%) and the mean liver weights in the high dose animals were significantly higher than controls at 6 months (120%). At other times there were increases in liver weights and liver-to-body weight ratios in one or more of the treated groups, but none were statistically significant. The authors state that these results suggest that there may be a marginal effect of the chemical on mean hepatic weights in female rats.

At 12 months, the mean testicular weights and testes-to-body weight ratios in all groups of the treated animals were lower than controls, but only the mean testicular weights in the high dose animals were statistically significant (88% of controls). At 17 months, none of the means for the treated animals were statistically significant, but the means were slightly lower than controls for high dose males (88% and 90% for testes and testes-to-body weight ratio, respectively). At termination, the mean testicular weights of both mid- and high dose groups (77% and 75% of controls, respectively) and the mean testes-to-brain weights of the high dose group were significantly lower than controls (79% of controls). The results suggest an effect at the high dose for testicular weights and a possible marginal effect at the mid-dose. No effects were observed in any of the other organ weights. The changes observed were considered to be random occurrences.

12. Histopathology:

a. Nonneoplastic lesions: With the exception of testicular lesions, all nonneoplastic microscopic findings in the study were considered to be incidental to treatment. Incidental lesions were normally found in the liver, kidneys, lung, heart, spleen, adrenals, pancreas, and thyroid gland. The incidences of unilateral and bilateral testicular atrophy are summarized in Table I. The incidence was similar between control and low dose animals, but was increased in mid- and high dose animals. Microscopically, the seminiferous tubules were frequently devoid of spermatid formation and germinal epithelial cells. In several cases, only Sertoli cells remained. In addition to atrophy, microscopic findings included polyarteritis, periarteritis, mineralization of arterioles and occasionally a sperm granuloma, an interstitial cell tumor, scrotal varicocele, orchitis, oligospermatogenesis and bilateral seminiferous tubule atrophy.

b. Neoplastic lesions: No neoplastic lesions were observed that were considered to be related to treatment. Neoplastic lesions were generally observed either in low incidence in all groups, including controls or only in an occasional animal. Tumors that were seen included hepatocellular adenomas; pituitary adenomas

and chromophobe adenomas; mammary gland adenomas, adenocarcinomas and fibroadenomas and islet cell adenomas of the pancreas (see Table II).

13. Quality Assurance Measures: Quality assurance measures were followed and the study was audited on a monthly basis. The report was signed by the Quality Assurance Manager.

C. DISCUSSION: There were several places in which the procedures in this study deviated from the EPA Testing Guidelines: 1) For the clinical chemistry studies, the same animals were not used for each time point. These animals were sacrificed at each time point. 2) Histopathology on gross lesions in the low and mid-dose animals sacrificed at 3, 6 and 17 months was not conducted. Microscopic examinations were conducted on gross lesions in these dose groups at the 12 month sacrifice, at termination and on all animals that either died or were sacrificed in extremis during the study. 3) The lungs and the kidneys were not examined in the low and mid-dose groups at the 3 and 6 month sacrifice times.

Point number 1 is not considered to be one that would significantly affect the outcome of the study. Since microscopic examinations were conducted on gross lesions and the specific organs mentioned above in point 3 from both the low and mid-dose groups at other sacrifice times, and since the results were negative except for testicular effects, these points are also not considered to be significant to the outcome of this study. Therefore, this study is classified as CORE GUIDELINE for the chronic portion of the study. The NOEL for the study is 2.49 mg/kg/day based upon testicular atrophy in males and the LOEL is 9.84 mg/kg/day. In females, a significant increase in hepatic mixed function oxidase activity was observed in both the mid- and high dose groups at 3 months, the mean liver-to body-weight ratio was elevated at 3 months and the mean liver weight was elevated at 6 months. These effects are most likely an adaptive response. Therefore, it appears that the chemical has a minimal effect on females at the dose levels tested.

The chemical was not oncogenic under the conditions of the study. The study is classified as CORE SUPPLEMENTARY for the oncogenic portion of the study because the Toxicology Branch (TB) does not believe that the top dose level tested was sufficiently high enough. It does not appear that the Maximum Tolerated Dose (MTD) was reached. Other than testicular atrophy, there was a marginal effect on liver weights in females at the highest dose level tested. Body weights were marginally decreased in males, but food consumption was also less than controls. In addition, an increase in liver mixed function oxidase was also observed. These effects are not considered severe enough to establish that the highest dose level tested (800 ppm) approached the MTD. Testicular atrophy is not likely to be life-threatening, and thus, higher dose levels could have been used. In addition, this lesion did not appear in the rat subchronic study. The dose levels selected for the chronic study appeared to be selected on the basis of increase in liver weights and liver enzyme induction.

The dose levels selected for the rat subchronic study were approximately 10, 30, 100, 300, 1000, 3000, 10,000 and 30,000 ppm. No effects were observed with dose levels up to and including 100 ppm. At 300 ppm, increases in liver mixed function oxidase activity were observed. At 1000 ppm, increases in the mean relative liver weights in females and accentuated liver architecture (seen grossly but nothing was noted in the microscopic examinations) were observed in addition to what was seen at 300 ppm. At 3000 ppm, increases in kidney and liver weights and SGOT were observed as well as that noted for 1000 ppm. In addition, hepatocellular hypertrophy was seen in the majority of the animals and hepatocellular necrosis was seen in 1/10 males and 3/10 females. Pigmentation of the convoluted tubular epithelium of the kidney was also observed at this dose level in males only. At 10,000 ppm, the effects noted at 3,000 ppm were observed (hepatocellular necrosis was seen in only 1 animal of each sex) as well as vacuolated swollen hepatocytes, blood effects (indications of red cell destruction and compensatory red cell production and hemosiderosis), increases in liver enzymes and BUN, Kupffer cell pigmentation. All of the animals which received 30,000 ppm died prior to the end of the testing period. Based upon the effects noted in the subchronic study in rats, TB believes that higher dose levels should have been used in the rat chronic/oncogenicity study.

TABLE I
INCIDENCE OF UNILATERAL AND BILATERAL TESTICULAR ATROPHY

	Control	Low	Mid	High
		12-Month Sacrifice		
Bilateral	0/20	0/19	1/20	3/20
Unilateral	0/20	1/19	0/20	0/20
		17-Month Sacrifice		
Bilateral	2/18	2/18	0/18	4/18
Unilateral	2/18	2/18	0/18	1/18
		Terminal Sacrifice		
Bilateral	2/17	1/19	5/20	12/22
Unilateral	2/17	3/19	6/20	2/22
		Animals That Died or Were Sacrificed Moribund		
Bilateral	1/35	4/35	10/32	12/30
Unilateral	6/35	4/35	5/32	5/30
		Total Incidence of Testicular Atrophy Across All Groups*		
Bilateral	5/110	7/110	16/110	31/110
Unilateral	10/110	10/110	11/110	8/110

* Including 3 and 6 month sacrifices (10 animals apiece, except low dose at 3 months had only 9 animals).

Table II. Incidence of Neoplastic Microscopic Findings (Summary of Significant Lesions)

Organ (Lesion)	Males				Females			
	Control	Low	Mid	High	Control	Low	Mid	High
12-Month Sacrifice								
Liver								
Hepatocellular Adenoma	0/20	0/19	0/20	0/20	0/19	0/20	0/20	2/20
Pituitary								
Adenoma	0/20	1/18	0/20	0/19	4/19	3/20	3/20	3/20
Mammary Gland								
Adenocarcinoma	0/1	0/1	0/0	0/4	4/10	2/3	2/5	2/17
17-Month Sacrifice								
Pituitary								
Chromophobe Adenoma	5/6	0/0	0/0	3/3	2/3	0/0	0/0	0/0
Adenoma	1/6	0/0	0/0	0/3	1/3	0/0	0/0	0/0
Mammary Gland								
Adenoma	0/1	0/0	0/0	0/0	2/5	0/0	0/0	1/3
Terminal Sacrifice								
Liver								
Hepatocellular Adenoma	0/17	1/19	0/19	0/22	0/24	0/20	0/20	1/25
Pancreas								
Islet Cell Adenoma	3/17	0/5	0/3	2/22	1/24	1/13	0/13	1/25
Pituitary								
Chromophobe Adenoma	12/17	1/5	3/4	7/22	14/23	18/19	10/15	18/25
Mammary Gland								
Adenocarcinoma	0/2	0/1	0/0	0/3	1/23	0/15	1/11	0/25
Fibroadenoma	0/2	1/1	0/0	0/3	10/23	11/15	6/11	11/25
Adenoma	0/2	0/1	0/0	0/3	3/23	6/15	4/11	2/25

Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

Budd 1/12/88

006580

DATA EVALUATION REPORT

STUDY TYPE: Range-Finding Dog (2-4 weeks) TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266078

TEST MATERIAL: RH-3866

SYNONYMS: RH-53,866; Myclobutanil; Systhane; Rally

REPORT NUMBER: 83R-078

SPONSOR: Rohm & Haas Co., Philadelphia, PA

TESTING FACILITY: Rohm & Haas Co., Toxicology Dept., Spring House, PA 19477

TITLE OF REPORT: RH-3866: A Dietary Range-Finding Study in Dogs

AUTHOR(S): P.R. Goldman & H.F. Emmons

REPORT ISSUED: October 8, 1986

IDENTIFYING VOLUME: Volume 4 of 47

CONCLUSION: The NOEL for the study is 250 ppm due to reduction in food consumption and bodyweights at 1000 ppm in females and at 4000 ppm in both sexes. The decreases in body weight were probably due to the low palatability of the diet at the levels where the effect was observed. No other clinical signs were observed.

Classification: CORE SUPPLEMENTARY

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile
Description: brown-colored solid
Batch #(s), Other #(s): Sample No. TD 83-087, Lot # LSPL 83-0017E
Purity: 84.5%
Source: Rohm & Haas

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): male and female Beagle dogs
Age: 5 months (6 months at start of dosing)
Source(s): Marshall Research Animal (North Rose, NY)

3. Procedure:

a. Dietary Preparation (if applicable): Aliquots of test material liquified in water bath (65°C), stirred, weighed and dissolved in acetone. Material was then mixed thoroughly in diet and acetone evaporated.
Frequency of preparation: Weekly
Storage conditions: Room temperature
Stability Analyses: measured on each dietary concentration at end of weekly dosing period.
Homogeneity Analyses: on first batch of diets, samples from top, middle and bottom of each dietary concentration analyzed for active ingredient.
Concentration Analyses: Only done with samples collected for stability and homogeneity analyses.

b. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Time Period Administered weeks	Number of Animals male	female
Contr.	0	4	2	2
1	50	4	2	2
2	250	4	2	2
3	1000	4	2	2
4	4000	2	2	2

c. Clinical Observations and Mortality: Each dog observed twice daily and once on weekends. Physical exams conducted weekly.

d. Body Weight Determinations: Weekly

e. Food and/or Water Consumption: daily

65

f. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals):
twice during pretest period (days -2 and -9) and on days 12 and 28 of treatment.
The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*		Mean corpustular HGB (MCH)
x	Hemoglobin (HGB)*		Mean corpustular HGB conc.(MCHC)
x	Leukocyte count (WBC)*		Mean corpustular volume (MCV)
x	Erythrocyte count (RBC)*	x	Red cell morphology
x	Platelet count*		
	Total plasma protein (TP)		
x	Leukocyte differential count*		

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X		X	
	Electrolytes:		Other:
x	Calcium*	x	Albumin*
	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorus*	x	Cholesterol*
	Potassium*	x	Globulins
	Sodium*	x	Glucose*
	Enzymes:	x	Total bilirubin*
x	Alkaline phosphatase	x	Total protein*
	Cholinesterase		Triglycerides
	Creatinine phosphokinase*	x	A/G ratio
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
x	Gamma glutamyl transferase		

g. Gross Necropsy:

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations:

all

h. Histopathology:

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination:

None. The heart, liver, kidneys and gross lesions were collected and preserved for possible future examination.

i. Statistical Analyses: Data reported on an individual animal basis. No statistical analyses were conducted.

B. RESULTS:

1. Dietary Preparation: Recovery for the amount of test chemical in the diet (1 day aged) averaged 92 +/- 11%. Dose levels from homogeneity samples ranged from 82-109% with an average of 93% of the theoretical dose.

2. Clinical Observations and Mortality: No deaths were observed and no treatment-related clinical signs of toxicity were observed at any dose level. Clinical signs of soft stool, mucus or blood in the stool, diarrhea and/or emesis were noted throughout all groups (including controls) both before and during all weeks of treatment. No dose-related responses were noted.

3. Body Weight Determinations: Body weights were decreased during both weeks of exposure at the 4000 ppm level in both sexes. At the end of 2 weeks the body weights of these animals were approximately 70% of the control values. There was a slight decrease in the bodyweight gains of the female animals at the 1000 ppm level during the first week. The gain in these animals was comparable to the controls in the last three weeks of the study. No other differences in body weight gains were noted for any of the other groups when compared to controls.

4. Food and/or Water Consumption: At 4000 ppm, a reduction in food consumption was observed in both sexes. Food consumption in this group was less than 4% of the control value during the second week. Food consumption of females in the 1000 ppm group was slightly decreased during the first week of treatment but was comparable to controls during the final three weeks of treatment. The food consumption in all the other groups was comparable to controls. Granular beef boullion was added to the diet in the highest dose group during week 2, however, consumption did not increase. Due to the lack of toxic signs, the authors judged the reduction in food consumption to be due to low palatability.

5. Hematology: No treatment-related changes were observed.

6. Clinical Chemistry: No treatment-related changes were observed. Decreases in glucose levels at the 4000 ppm dose level were considered to be due to the reduction in food intake.

7. Gross Pathology: No compound-related lesions were observed in any of the dose groups. In only 1 instance did an observation occur at more than 1 time: a red area on the spleen was observed in the 2 1000 ppm male dogs. This was not considered to be treatment-related.

8. Histopathology: Not done.

9. Quality Assurance Measures: The report and original data have been reviewed for adherence to GLP's by the Quality Assurance Unit of the Rohm & Haas Company.

C. DISCUSSION: This study is a range-finding study for the chronic dog study that was to be conducted on this chemical. Due to the nature of the study and the purpose of its design, it can only considered to be CORE SUPPLEMENTARY.

Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

Budd 11/12/88

006580

DATA EVALUATION REPORT

STUDY TYPE: Chronic Feeding Nonrodent (Dog) (83-1)

TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266088

TEST MATERIAL: RH-3866

SYNONYMS: Systhane, Rally, Myclobutanil, RH-53,866

REPORT NUMBER: 84R-078

SPONSOR: Rohm & Haas Co., Philadelphia, PA

TESTING FACILITY: Rohm & Haas Co., Toxicology Dept., Spring House, PA

TITLE OF REPORT: RH-3866: One Year Dietary Study in Beagle Dogs

AUTHOR(S): P.R. Goldman, J.C. Harris and J.D. Frantz

REPORT ISSUED: October 15, 1986

IDENTIFYING VOLUME: Volume 14 of 47

CONCLUSION: NOEL 100 ppm (3.09 mg/kg/day for males and 3.83 mg/kg/day for females) based upon hepatocellular hypertrophy. Supporting effects in organ weights and clinical chemistry observed. LOEL 400 ppm (14.28 mg/kg/day for males and 15.68 mg/kg/day for females)

Classification: CORE MINIMUM because full histopathology examinations were not submitted on the mid- and low dose levels.

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-4-chlorophenyl-1-H-1,2,4-triazole-propanenitrile
Description: white solid
Batch #(s), Other #(s): Lot No. 83159-7, Sample No. (TD No.) 84-063
Purity: 91.4%
Source: Rohm & Haas

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): male and female beagle dogs
Age: 5 months at start of test
Source(s): Marshall Research Animals (North Rose, NY)

3. Procedure:

a. Dietary Preparation (if applicable): RH-3866 was heated until liquified, stirred, weighed, dissolved in acetone and blended with the feed. The acetone was evaporated off.

Frequency of preparation: weekly

Storage conditions: Stored at room temperature in jars (one jar per weekly diet preparation)

Stability Analyses: a sample from each weekly preparation was taken for analysis. Select samples were analyzed.

Homogeneity Analyses: The first time the diets were prepared, samples were taken from the top, middle, and bottom of each dietary concentration and submitted for analysis.

Concentration Analyses: This was done in connection with the stability and homogeneity analyses.

b. Basis For Selection of Dosage Levels:

Doses selected on the basis of a one-month range-finding study.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 12 months male	female
Contr.	0	6	6
1	10	6	6
2	100	6	6
3	400	6	6
4	1600	6	6

d. Procedures for Studies Other Than Feeding and/or Additions, Changes in Feeding Study: Control and test diets were offered for same two hours per day.

e. Clinical Observations and Mortality: Dogs were observed daily for clinical signs of toxicity. Physical exams conducted weekly for first month and biweekly for remainder of treatment period.

f. Body Weight Determinations: Weekly

g. Food and/or Water Consumption: daily. Mean feed consumption per group calculated weekly.

h. Ophthalmological Examinations (if applicable): Conducted during pre-test period and during weeks 26 and 52 of treatment period.

i. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals):
weeks -2, -1, 13, 25, 39 and 53

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	x	Mean corpustular HGB (MCH)
x	Hemoglobin (HGB)*	x	Mean corpustular HGB conc.(MCHC)
x	Leukocyte count (WBC)*	x	Mean corpustular volume (MCV)
x	Erythrocyte count (RBC)*	x	Red blood cell morphology
x	Platelet count*		
	Total plasma protein (TP)		
x	Leukocyte differential count*		

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X		X	
	Electrolytes:		Other:
x	Calcium*	x	Albumin*
	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorus*	x	Cholesterol*
	Potassium*	x	Globulins
	Sodium*	x	Glucose*
	Enzymes:	x	Total bilirubin*
x	Alkaline phosphatase	x	Total protein*
	Cholinesterase		Triglycerides
	Creatinine phosphokinase*	x	A/G ratio
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
x	Gamma glutamyl transpeptidase		

3) Urinalysis:

Collection times for urine (including # of animals):
On each of 2 consecutive days at weeks -4 (all animals), 25 and 51 weeks (controls and high dose)

The following CHECKED (X) parameters were examined:

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

j. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations:

None were sacrificed and none died prior to completion of the study.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations:

All animals in all dose groups.

k. Histopathology:

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination:

All animals from high dose and control groups; liver, gallbladder and testes in all dogs from all dose groups. Tissues were preserved from all animals from all dose groups for possible future examination.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

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

l. Statistical Analyses: Distributions of all continuous data were inspected for normality and homogeneity of variance across treatment groups. Analyses of variance were used when needed. Duncan's multiple range test was used on some data as well as T-tests.

B. RESULTS:

1. Dietary Preparation: Average dose levels ranged between 97-111% of the theoretical dose levels, with an overall average of 103%.

2. Clinical Observations and Mortality: No deaths were observed and no clinical signs of toxicity were noted at any of the dose levels throughout the study.

3. Body Weight Determinations: The body weights of male dogs at the highest dose level were significantly decreased following one week of treatment but were similar to control values throughout the remainder of the study. The mean body weights of female dogs at this dose level were significantly less than the control values for the first 5 weeks of the study. No treatment related changes were noted in the body weights of either sex at any of the other dose levels.

4. Food and/or Water Consumption: Food consumption of female dogs fed the highest dose level was consistently below that of the controls throughout the study. Food consumption of males at the highest dose level was below that of the controls during the first week but was similar to controls during the remainder of the study. No differences were observed in the food consumption of the other groups when compared to controls.

5. Ophthalmological Examinations: No ophthalmological abnormalities were seen in any of the treated dogs.

6. Hematology: A slightly decreased number of red blood cells (RBC), an increased number of platelets, an increase in mean cell hemoglobin and an increase in the mean corpuscular volume were observed in male dogs at the highest dose level throughout the treatment period. A slight increase in the mean cell hemoglobin was observed in male dogs at 400 ppm. No other treatment related changes (other than spurious differences) were observed in any of the other parameters or in any of the other dose groups.

7. Clinical Chemistry: Increases in inorganic phosphorus and alkaline phosphatase and a decrease in serum albumin were observed in both sexes at the highest dose level. Alkaline phosphatase was also increased in females at 400 ppm. SGPT was increased in males and GGT was increased in females at the highest dose level (1600 ppm). These changes were consistent throughout the treatment period. No other consistent changes were noted in any of the other parameters or in any of the other dose groups.

8. Urinalysis: No treatment related changes were noted in any of the treated groups.

9. Gross Pathology: Gross changes were observed in the livers of high dose dogs of both sexes. These changes consisted of enlargement and/or accentuated lobular architecture (1 male and 3 females). Other changes were considered incidental and were found in all groups, including controls. Frequent changes included reddened portions of the intestinal tract, thickened and reddened mammary glands (females only) and distended uteri (females only).

10. Organ Weights: Increased absolute and relative liver weights were observed in both sexes at 1600 ppm and in females at 400 ppm. No other observed changes in organ weights were considered to be related to treatment. The statistically significant increase in absolute liver weight of 100 ppm female dogs is considered to be due to the larger size of the dogs in this group when compared to control dogs and the statistically significant increase in relative liver weights in the 10 ppm dogs is considered to be due in part to the smaller size of the dogs at this dose level.

11. Histopathology:

    a. Nonneoplastic lesions: Compound-related nonneoplastic lesions were observed in the livers of both sexes of dogs from the 400 ppm and from the 1600 ppm dose groups. These lesions included minimal to mild hepatocellular hypertrophy in 1/6 of the 400 ppm male dogs and in 5/6 of the 1600 ppm male dogs and mild to moderate hepatocellular hypertrophy in 2/6 of the 400 ppm female dogs and in 6/6 of the 1600 ppm female dogs. The hypertrophy was characterized by cells with large amounts of pale, eosinophilic, finely granular cytoplasm; in a few more severely affected livers it was noted to be almost panlobular in distribution. "Ballooned" hepatocytes or expanded hepatocytes with large clear cytoplasmic spaces, sometimes containing only a few strands of pink cytoplasm were sporatically observed in some of the more severely affected high dose females. These cells were thought to represent severely hypertrophied, possibly degenerating, hepatocytes. No changes in the liver related to treatment were noted in any of the animals in the lower dose levels. Table 1 summarizes the changes noted in the livers of the treated animals. No treatment related changes were observed in the testes of any of the male animals. Incidences of lesions in treated animals were similar to controls at all dose levels. Changes in other organs were considered to be incidental and not related to treatment.

    b. Neoplastic lesions: No neoplastic lesions were observed in any of the animals.

12. Quality Assurance Measures: The report and the original data from the study were reviewed for adherence to the GLP's and the study was audited a number of times throughout the pretreatment and treatment phases. The report is signed by the Quality Assurance Unit.

C. DISCUSSION: This study is in general well conducted. There is, however, one important item missing: a full microscopic examination was not conducted on the mid- and low dose animals. Full microscopic examinations on all animals in nonrodent studies are required by the EPA Guidelines. In addition to incomplete microscopic examinations, there were a few minor missing items in the hematology summary (no mention of the statistically significant increases in mean cell hemoglobin in the top two dose levels and an increase in mean corpuscular volume at the top dose level). The study is CORE MINIMUM because complete microscopic examinations were not submitted on the mid- and low dose levels. In this case, the study was excepted because no other effects were seen in any of the other chronic studies except testicular effects and the testes were examined microscopically at all dose levels in the study. It is not likely that complete microscopic examinations at all dose levels would change the outcome of the study. The NOEL is 100 ppm (3.09 mg/kg/day for males and 3.83 mg/kg/day for females) based upon hepatocellular hypertrophy and the LOEL is 400 ppm (14.28 mg/kg/day for males and 15.68 mg/kg/day for females). Supporting effects were observed as well.

RH-3866: ONE YEAR DIETARY TOXICITY STUDY IN BEAGLE DOGS
PROTOCOL NO. 84P-203 REPORT NO. 84R-078

Table 1 Summary Of Histopathology Observations

	Males					Females				
Group:	1	2	3	4	5	1	2	3	4	5
RH-3866 Technical: ppm	0	10	100	400	1600	0	10	100	400	1600
Number of Animals Per Group	6	6	6	6	6	6	6	6	6	6
Tissue/Microscopic Observations										
Liver										
Number Examined	6	6	6	6	6	6	6	6	6	6
Number Normal	3	1	2	4	0	1	2	2	1	0
hepatocellular hypertrophy, centrilobular	-	-	-	1	5	-	-	-	2	6
congestion	1	-	-	-	1	-	-	-	-	-
pigmentation, multifocal	1	1	-	-	-	-	-	-	-	-
mononuclear cell infiltrate, periportal, multifocal	2	-	1	-	-	2	-	-	2	1
eosinophils, periportal, multifocal	1	-	-	-	-	-	-	-	-	-
mononuclear cell infiltrate, central vein, multifocal	1	1	3	-	1	-	3	-	1	1
hepatic cord atrophy, subcapsular	1	-	-	-	1	-	-	-	-	-
mononuclear cell infiltrate, parenchymal, multifocal	-	5	3	1	-	4	2	4	4	5
hepatitis, acute, multifocal	-	-	-	-	-	-	-	-	-	1
"ballooned" hepatocytes, centrilobular	-	-	-	-	-	-	-	-	-	4
vacuolization, periportal, multifocal		-	-	-	-	-	-	-	1	-

Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

Budd 11/3/88

006580

DATA EVALUATION REPORT

STUDY TYPE: Repeated Dose Dermal Toxicity - 4 Weeks (82-2)

TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266080

TEST MATERIAL: RH-3866 40W and RH-3866 2EC

SYNONYMS: Rally, Systhane, Myclobutanil, Nova

REPORT NUMBER: 85R-240

SPONSOR: Rohm & Haas, Philadelphia, PA

TESTING FACILITY: Rohm & Haas, Toxicology Dept., Spring House, PA

TITLE OF REPORT: RH-3866: 4-Week Dermal Toxicity Study in Rats

AUTHOR(S): R. Bonin and G.A. Hazelton

REPORT ISSUED: 8/29/86

IDENTIFYING VOLUME: Volume 6 of 47

CONCLUSION: The systemic NOEL for both the 2EC and 40WP formulations is 100 mg a.i./kg/day (HDT) and the NOEL for skin irritation is 10 mg a.i./kg/day.

Classification: CORE MINIMUM for both the 2EC formulation and the 40WP formulation.

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile (active ingredient)

Description: RH-3866 2EC is an amber liquid and RH-3866 40WP is a beige powder

Batch #(s), Other #(s): RH-3866 2EC: lot # EG-0807-1, TD# 85-109; RH-3866 40WP: Lot # EG-0809-1, TD# 85-110

Purity: RH-3866 2EC: 24.99% a.i.; RH-3866 40WP: 41.36% a.i.

Source: Rohm & Haas

Vehicle (if applicable): RH-3866 2EC formulation blank

Positive Control(s) (if applicable): N/A

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female Sprague-Dawley Crl-CD® BR VAF/Plus™ albino rats
Age: 8 weeks
Weight(s): 250-290g (males), 166-203g (females)
Source(s): Charles River Breeding Laboratories, Lakeview Facility, Nutley, N.J.

3. Procedures:

Male and female rats were assigned to 6 groups, 6 animals of each sex per group. Two formulations were tested and two control groups were run. The first formulation, RH-3866 2EC was tested at 3 dose levels, 1, 10, and 100 mg a.i./kg. The dose levels were selected on the basis of results from a pilot skin irritancy study. A control group containing the 2EC formulation without the technical material was also run. The second formulation, RH3866 40 WP was only tested at 1 dose level, 100 mg a.i./kg (the pilot study indicated no irritation at this level). The second control group was treated with only distilled water (the vehicle for the 40 WP formulation). Prior to initial treatment, the hair on the back of each animal was clipped free of hair and reclipped as required throughout the study. Each rat was fitted with a cardboard collar in order to prevent oral ingestion of the material. All the dosing solutions were prepared by weighing out the appropriate material and diluting to the proper concentration with distilled water. The test materials were applied at a constant volume of 1.5 ml/kg, based upon the most recent boyweight of the animal. Bodyweights were recorded weekly. The application sites remained unoccluded throughout the study. Following the 6-hour exposure period, the application sites were wiped gently with water-moistened paper tissues. Rats were treated once a day (Monday through Friday) for a total of 19-20 days of treatment over a 4-week period.

Each animal was observed twice daily on treatment days and once daily on weekends for clinical signs of toxicity. Physical examinations were conducted weekly. Skin irritation was evaluated daily on treatment days, immediately before treatment. The degree of irritation was scored according to the grading system of Draize, et al. Hematology and clinical chemistry evaluations were performed on blood samples obtained 24 hr. after the last treatment. The following parameters were measured for hematology and clinical chemistry.

1. Hematology:

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	x	Mean corpustular HGB (MCH)
x	Hemoglobin (HGB)*	x	Mean corpustular HGB conc.(MCHC)
x	Leukocyte count (WBC)*	x	Mean corpustular volume (MCV)
x	Erythrocyte count (RBC)*		
x	Platelet count*		
	Total plasma protein (TP)		
x	Leukocyte differential count*		

2. Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X	Electrolytes:
	Calcium*
	Chloride*
	Magnesium*
	Phosphorus*
	Potassium*
	Sodium*
	Enzymes:
x	Alkaline phosphatase
	Cholinesterase
	Creatinine phosphokinase*
	Lactic acid dehydrogenase
x	Serum alanine aminotransferase (also SGPT)*
x	Serum aspartate aminotransferase (also SGOT)*

X	Other:
x	Albumin*
x	Blood creatinine*
x	Blood urea nitrogen*
x	Cholesterol*
x	Globulins
x	Glucose*
x	Total bilirubin*
x	Total protein*
x	Triglycerides
x	A/G Ratio (albumin/globulin)

At the end of the 4 week test period, the rats were necropsied and grossly examined, organ weights were recorded and tissues were taken for histopathological examination. CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

X	Digestive system
	Tongue
x	Salivary glands*
x	Esophagus*
x	Stomach*
x	Duodenum*
x	Jejunum*
x	Ileum*
x	Cecum*
x	Colon*
x	Rectum*
xx	Liver*
	Gall bladder*
x	Pancreas*
	Respiratory
x	Trachea*
x	Lung*

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

X	Neurologic
x	Brain*
x	Periph. nerve*
x	Spinal cord (3 levels)*
x	Pituitary*
x	Eyes (optic n.)*
	Glandular
xx	Adrenals*
	Lacrimal gland
x	Mammary gland*
x	Parathyroids*
x	Thyroids*
	Other
x	Bone*
x	Skeletal muscle*
x	Skin
x	All gross lesions and masses

Histopathologic examinations were performed on the testes, liver and kidneys of all rats from the two controls, the high-dose 2EC group and the 40 WP group. The skin (treated and untreated) of all rats from all groups was evaluated. In addition, all gross lesions from animals in all groups were evaluated.

Distributions of all continuous data were inspected for normality and homogeneity of variance across treatment and sex groups. Transformation of the data was conducted when necessary. The overall treatment effect was assessed by a two-way analysis of variance. Other statistical methods were utilized depending upon the data being examined.

B. RESULTS:

No clinical signs of toxicity related to treatment were observed in any of the treated groups. In addition, no deaths were reported for any of the groups. Red stained eyes and muzzle were observed in all groups and was believed to be associated with the cardboard collars. Other effects were observed in all groups and were believed to be either related either to the cardboard collars or to the metal ears tags, and not to treatment.

In males, slight skin irritation was observed in the highest dose group of the 2EC formulation on days 2 through 25 of the study, and moderate irritation was observed on days 26 through 29 of the study (see summary skin irritation table). In females, slight skin irritation was sporadically observed in this dose group on days 2 through 29 of the study. Females treated with the 2EC vehicle control solution exhibited slight skin irritation on days 2 and days 20 through 29 of the study. In males, slight skin irritation was observed in the vehicle control group on day 2. The findings indicate that irritation was observed only in the high dose 2EC group and in the vehicle control group which has the same concentrations of inerts as the high dose group. None of the other treated groups indicated any signs of dermal irritation.

No biologically significant differences between the treated and control groups were observed for body weight gain, hematological or clinical chemistry values, or organ weights. The gross necropsy indicated changes in the skin in the 2EC vehicle control group, the high-dose 2EC group and the 40WP group. Eschar was noted in 1 male and 2 females in the 2EC vehicle control group, 3 males and 4 females in the high-dose 2EC group, and 1 male in the 40 WP group. Desiccation was observed in 1 male and 2 females in the 2EC vehicle control group, 1 male and 5 females in the high-dose 2EC group, and 4 males and 2 females in the 40 WP group. One male in the high-dose 2EC group exhibited pinpoint red discoloration, while another male from this group showed thickening of the skin (see summary table).

Microscopic changes related to treatment were observed only in the treated skin. No microscopic differences between the testes of the treated animals (high dose 2EC and 40WP groups) and the control animals were observed. A majority of the rats in the high-dose 2EC group (both sexes) exhibited significant microscopic changes in the skin including epidermal necrosis, epidermal thickening, and/or subacute/chronic inflammation of the dermis. Similar changes were observed in the 2EC vehicle control group but the findings occurred at a lower incidence and the severity of the changes were decreased. The changes in the mid- and low-dose groups were comparable to the vehicle control groups (i.e., a few animals exhibited a minimum degree of chronic inflammation and/or epidermal thickening and one animal was noted to have parakeratosis). A majority of the rats in the 40 WP group exhibited a minimal to mild degree of chronic inflammation and epidermal thickening and one male

81

and one female each was noted to have eschar formation (see summary table). No other microscopic changes related to treatment were observed.

C. DISCUSSION:

For the 2EC formulation, the NOEL for systemic effects is 100 mg a.i./kg/day, the highest dose level tested. The NOEL for skin irritation is 10 mg a.i./kg/day. The NOEL for systemic effects for the 40WP formulation is also 100 mg/kg/day, the only dose tested. There are several items in the conduct and design of this study which deviate from the EPA testing Guidelines. The first is that unoccluded dressings were used on the animals. The investigators placed collars on the animals instead, in order to prevent ingestion of the test material. The second item is that in the clinical chemistry studies, no electrolyte values were measured. The Guidelines suggest measurement of several electrolytes, including sodium, potassium and chloride. Lastly, the 40WP formulation was only tested at one dose level. The level chosen was equivalent in the amount of active ingredient tested as the highest dose level tested with the 2EC formulation. However, since no systemic effects were observed at the dose level applied and since no systemic effects were observed at any of the dose levels tested with the 2EC formulation, this study is graded CORE MINIMUM for both the 2EC formulation and the 40WP formulation.

Summary of Gross and Microscopic Observations for Skin

Gross Necropsy Results

	Males						Females					
Group	1	2	3	4	5	6	1	2	3	4	5	6
Dose (mg/kg)	0	0	1	10	100	100	0	0	1	10	100	100
Compound	Water Control	Control Vehicle	2EC	2EC	2EC	40WP	Water Control	Control Vehicle	2EC	2EC	2EC	40WP
(# tissues examined)	6	6	6	6	6	6	6	6	6	6	6	6
Neck - alopecia	6	3	3	2	3	1	1	2	1	1	2	-
Treated												
Eschar	-	1	-	-	3	1	-	2	-	-	4	-
desiccation	-	1	-	-	1	4	-	2	-	-	5	2
thickened	-	-	-	-	1	-	-	-	-	-	-	-
discoloration (focal/ multifocal)	-	-	-	-	1	-	-	-	-	-	-	-

Microscopic Examinations

Untreated Skin												
Chronic inflammation (dermis)	-	1	-	-	-	2	1	2	-	-	-	2
Epidermal thickening	-	-	-	-	-	1	-	-	-	-	-	-
Subacute inflammation (dermis)	-	-	-	1	-	-	-	-	-	-	-	-
Treated Skin												
Chronic inflammation	-	3	2	2	4	6	1	5	2	-	5	4
Epidermal thickening	-	2	-	1	5	6	-	3	-	-	6	3
Eschar	-	-	-	-	4	1	-	2	-	-	3	1
Epidermal necrosis	-	-	-	-	2	-	-	1	-	-	4	-
Subacute inflammation	-	-	-	-	2	-	-	1	-	-	1	-
parakeratosis	-	-	-	-	-	-	-	-	1	-	-	-

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Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

Budd 1/12/88

006580

DATA EVALUATION REPORT

STUDY TYPE: Teratogenicity (83-3) - Rabbit TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266097

TEST MATERIAL: RH-53,866

SYNONYMS: Systhane, Rally, Nova

REPORT NUMBER: 83R-216

SPONSOR: Rohm and Haas Co., Philadelphia, PA 19477

TESTING FACILITY: Rohm and Haas Co., Spring House, PA 19477

TITLE OF REPORT: Range-Finding Teratology Study with RH-53,866 in Rabbits

AUTHOR(S): R.D. Costlow and W.W. Kane

REPORT ISSUED: October 31, 1984

IDENTIFYING VOLUME: Volume 23 of 47

CONCLUSION: Maternal and embryofetotoxicity NOEL's 100 mg/kg/day. Not a teratogen at dose levels up to 215 mg/kg/day, the highest dose level at which term fetuses were available.

Classification: Supplementary (range-finding study)

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: Alpha-Butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile
Description: solid
Batch #(s), Other #(s): Lot LSPL83-0017E, TD #83-087, TD #83-255
Purity: 84.5%
Source: Rohm and Haas
Vehicle (if applicable): Hi-Sil 233 (solid adsorbent), methylcellulose (liquid suspending agent)

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): New Zealand white rabbits
Age: Approximately 5 months
Weight: Over 3.0 kg
Source(s): Hazleton/Dutchland Farms, Denver, PA

3. Procedures:

Forty-five females were artificially inseminated with sperm from males of the same strain, same supplier. Forty-two of these animals were selected for the study. The inseminated females had been induced to ovulate by an intravenous injection of chorionic gonadotropin approximately 3 hours prior to insemination. The day of insemination was considered day zero of gestation. Administration of the test substance commenced on day 7 of gestation. The test substance was administered in a vehicle via oral intubation, using a constant dose volume of 5.0 ml/kg. The following dose levels were used: 0, 10, 31.6, 100, 215, 464, and 700 mg/kg/day, 6 animals/dose group. The animals were dosed through day 19 of gestation. The animals were examined daily for clinical signs of toxicity. Body weights were recorded for all surviving does on days 0, 4, 7, 11, 15, 20, 25 and 29 of gestation. The does were sacrificed on day 29 of gestation and the following items were examined and recorded: the number and uterine position of viable fetuses, nonviable fetuses, early resorptions and late resorptions. In addition, total implantations were tabulated and recorded and the number of corpora lutea on each ovary were recorded. All term fetuses were weighed and examined externally for malformations. Pre-term fetuses were examined where feasible. Appropriate statistical analyses were conducted where necessary.

B. RESULTS:

The Fertility Index (# pregnant/# inseminated) was 0.5 or greater for each group. There were 11 rabbits which were not pregnant; 1 in the 10 mg/kg group, 2 in each of the control and 100 mg/kg groups and 3 in the 464 and 700 mg/kg groups. 6/6 does in both the 700 and 464 mg/kg dose groups (3 pregnant in each of the 2 dose groups) died during gestation. The deaths were treatment-related. One death at the 100 mg/kg dose level occurred due to an intubation error. No other deaths were observed. One doe in the 215 mg/kg dose group aborted her litter. The authors state that the abortion was probably treatment-related (combination of maternal toxicity and embryofetotoxicity).

No treatment-related clinical signs of toxicity were observed in animals in the 100 mg/kg/day or less dose groups. Clinical signs of toxicity which appeared prior to death in the two top dose groups included irregular feces, exudate/discharge, ataxia, lethargy or prostration, purulent discharge from the nose or eyes, cyanosis, difficulty breathing, convulsions, paralysis, rapid breathing and red urine. At 215 mg/kg, clinical signs included increased incidences of irregular feces and red urine.

Only one animal survived to day 11 in the top two dose levels. This animal was in the 464 mg/kg dose group and showed dramatic weight loss by day 11 (statistically significant). At 215 mg/kg, the weight loss was significant by day 11 of gestation and maternal weights were significantly less than controls throughout dosing. Weight loss stopped when treatment was suspended.

Necropsy observations of the dams at the 215 mg/kg dose level and below were not different from controls. Treatment-related findings in the 464.4 mg/kg group were consistent with gastric irritation, agonal breathing and/or general antemortem malaise. The animals in the 700 mg/kg group died too quickly

for the evidence of toxic signs to be observed, however, evidence of agonal breathing was present.

Corpora lutea/litter, implantation sites/litter and implantation efficiency were within normal limits for all groups. In the two highest dose groups, although all the does died prior to sacrifice, gross examinations of the fetuses showed that they were normal, however, the authors state that it is likely that they would not have survived to full term due to the high incidence of resorptions at 215 mg/kg. Although statistical analysis of the Viability Index indicated no significant dose-related effect, the individual data indicated that RH-53,866 was embryotoxic at the 215 mg/kg dose level. Two of five litters were totally resorbed and a third doe resorbed 6/9 implantations. A reduction in viable fetuses/litter and increased resorptions/litter were apparent at this dose level. These effects were not observed at the lower dose levels. There were no dead fetuses in any litter in the study.

No significant differences were observed in mean fetal weights up to and including those in the 215 mg/kg dose level. However, when compared to the lower dose levels, a smaller litter size in the 215 mg/kg dose group did not reflect a proportionally elevated fetal weight. The authors stated that these data suggested a fetotoxic response at this dose level.

In conclusion, RH-53,866 was lethal to New Zealand White rabbits at doses of 464 and 700 mg/kg/day. 215 mg/kg/day was maternally toxic as well as embryotoxic, but the fetuses at this level appeared to be grossly normal upon necropsy. The NOEL was 100 mg/kg for maternal toxicity and embryofetotoxicity. The chemical did not appear to be a teratogen at dose levels up to 215 mg/kg/day, the highest dose level at which term fetuses were available.

C. DISCUSSION:

This study appears to be a well-run study for a range-finding study. It is CORE SUPPLEMENTARY.

Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

Budd 1/12/88

006580

DATA EVALUATION REPORT

STUDY TYPE: Teratogenicity (83-3) - Rabbit TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266098

TEST MATERIAL: RH-53,866

SYNONYMS: Systhane, Rally, Nova

REPORT NUMBER: 83R-217

SPONSOR: Rohm and Haas Company, Philadelphia, PA

TESTING FACILITY: Rohm and Haas Company, Toxicology Dept., Spring House, PA 19477

TITLE OF REPORT: Teratology Study with RH-53,866 in Rabbits

AUTHOR(S): R.D. Costlow and W.W. Kane

REPORT ISSUED: November 15, 1984

IDENTIFYING VOLUME: Volume 24 of 47

CONCLUSION: RH-53,866 was not teratogenic at any dose level up to 200.0 mg/kg/day, the highest dose level tested. The NOEL for maternal toxicity was 20.0 mg/kg/day and the fetoembryotoxic NOEL was 60.0 mg/kg/day. The A/D ratio is 1/3.

Classification: CORE MINIMUM

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazole)-1-propanenitrile
Description: amber viscous liquid/solid
Batch #(s), Other #(s): Lot # LAP-0298 and TD # 83-260
Purity: 90.4%
Source: Rohm and Haas
Vehicle (if applicable): HiSil 233 carrier suspended in 1% methylcellulose (A4C)

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): New Zealand White Rabbit (female)
Age: 5 months
Weight(s): 2.5-4.2 kg
Source(s): Hazleton Dutchland, Denver, PA

3. Procedures:

Five groups of 18 female rabbits were used for the study. The following dose levels were tested, based upon a preliminary range-finding study (summarized in a separate report): 0 (water control), 0 (Hi-Sil control), 20.0, 60.0 and 200.0 mg/kg/day a.i. (5 ml/kg b.w.). The test chemical was prepared with the solid adsorbent (carrier) Hi-Sil 233 and with 1.0% methylcellulose (vehicle). The dosing suspensions were prepared daily and samples were retained for analysis. Samples from the first, tenth and last dosing days were actually submitted for analysis. The male rabbits used in the study were of the same strain and from the same source as the females. These animals had been maintained at the facility for breeding purposes. The females were artificially inseminated and the day on which insemination occurred was considered to be day 0 of gestation. Three hours prior to insemination, the does were induced to ovulate with an intravenous injection of chorionic gonadotropin. The test chemical was administered by oral intubation on days 7 through 19 of gestation.

The does were observed for signs of clinical toxicity and mortality at least once daily (twice daily during the dosing period). Body weights were recorded on days 0, 4, 7, 11, 15, 20, 25 and 29 of gestation. On day 29 of gestation the does were sacrificed and the following items were examined and recorded: the number and uterine position of viable and non-viable fetuses, and early and late resorptions. In addition, total implantations were tabulated and recorded and the number of corpora lutea on each ovary was recorded.

Each live fetus was weighed and examined for external malformations and variations. Each was then dissected and internally sexed and examined for visceral malformations and variations. Each brain was examined through a mid-coronal incision and each heart was examined by a modification of the Staples method. All fetuses were skinned, fixed in 95% ethyl alcohol, mascerated in potassium hydoxide and stained with Alizarin red S for subsequent skeletal examination. Variations and malformations were tabulated for the fetuses.

Any data that suggested any treatment-related effects were statistically analyzed. Parametric and nonparametric procedures were used. The various indices (e.g. Fertility, Viability, etc.) were analyzed by the Jonkheere test for dose-related trends followed by pairwise comparisons of treated groups to separate and/or combined controls.

B. RESULTS:

Maternal Observations:

No signs of clinical toxicity were observed in the 20 and 60 mg/kg/day groups when compared to controls. In the 200 mg/kg/day group, increases in the following signs were noted: irregular feces, bloody urine, bloody urogenital or anal area and blood and/or aborted material in the drop pan. The authors state that the incidences of blood or aborted material in the drop pan in the 200 mg/kg group were related to the abortions or total resorptions. The incidences of blood in the pan at 20 and 60 mg/kg were related to abortions

which occurred in these groups. Two of 18 does in the 200 mg/kg group died due to intubation errors. No other deaths were observed in any of the other groups. The Fertility Index was 0.67 or greater for each group in the study and 0.76 overall. The following number of rabbits/group did not get pregnant: 5/18 (water control), 6/18 (Hi-Sil control), 4/18 (20 mg/kg), 3/18 (60 mg/kg) and 4/18 (200 mg/kg). There were no abortions in the controls and 1 each in the 20 and 60 mg/kg dose groups. These were considered to be incidental abortions and not related to treatment. The conceptuses recovered from these two abortions were early resorptions, suggesting that the fetuses died in utero prior to the abortion. Three does in the 200 mg/kg group aborted their litters. This incidence was considered by the authors to be related to treatment - probably a combined maternal toxicity and an embryofetotoxic effect. All the recovered conceptuses were early resorptions. See Table 7 for details.

No treatment-related difference in body weights was observed in does at the 20 mg/kg level. At 60 mg/kg, it appeared that maternal toxicity occurred with the first exposures to the test chemical, from which the does recovered and resumed a normal growth pattern at a reduced weight. At 200 mg/kg, maternal weight gain during treatment was significantly reduced.

There were no differences observed between any of the treated groups and the controls when the dams were examined at necropsy.

Ovarian, Uterine and Fetal Data

The number of corpora lutea/litter was not significantly different in any of the treated groups when compared to controls, although at 200 mg/kg the number was less (7.56 as opposed to 11.31 and 12.75 for controls). With the exception of the 200 mg/kg group, the numbers were higher than historical control values. The number of corpora lutea/litter for the 200 mg/kg group was within the historical control limits. In addition, the number of implantation sites/litter was within normal limits for all groups when compared to historical control data. There were no dead fetuses in any of the treated groups. In the 20 and 60 mg/kg groups there were no litters with more than 2 resorptions and no litters completely resorbed. The Viability Index for these two groups was not significantly different from combined control values. At a dose level of 200 mg/kg/day, when compared to controls, there was an increased incidence in the number of resorptions/litter, the number of litters with more than 2 resorptions and the number of litters totally resorbed. The Viability Index was significantly reduced when compared to controls. See Table 3 for details.

Litter size was significantly reduced at the 200 mg/kg level. At the other two dose levels, litters sizes were not different from controls. There were no statistically significant differences in fetal weights in any of the treated groups when compared to controls. The fetal sex ratio (# males/# females was not significantly different in any of the treated groups when compared to combined controls. The higher value (2.33) in the 200 mg/kg group was considered by the authors to be a random occurrence because the number was based upon a small number of litters. The ratios for the controls were 1.48 and 1.03 (water controls and vehicle controls, respectively). See Table 4 for details.

There were no external variations in any group. The frequencies of the soft tissue variations were not suggestive of an effect at any dose, not was there a dose-response. The frequencies across the groups were within the range of historical controls. There were no significant trends across control and treated groups for any skeletal variations. The percentage of fetuses with some of the more common variations tended to be higher in the 200 mg/kg group than in any of the other groups. This may be due to the fact that resorptions and abortions were so high in this group that the number of fetuses available for examination was approximately one-third of the numbers available in the other groups. The most common skeletal variations observed throughout the groups were: 13th rudimentary ribs (unilateral or bilateral), 13th full ribs (bilateral), 13th full rib with 13th rudimentary rib, and 27 presacral vertebrae. Other variations observed included: bent hyoid arches, accessory nasal bones, 25 presacral vertebrae, sternebra 5 and/or 6 unossified and sternebrae misaligned. No primary external malformations were noted for any of the test groups. Cardiomegally was found in one fetus in one litter at 20 mg/kg. This was considered to be a spontaneous occurrence. No treatment-related skeletal malformations were observed in any of the treated groups. Single occurrences were observed in different dose groups. These included fused skull bones, fused sternebrae, ribs with spherical enlargements, and scoleosis (with or without associated rib anomalies). When all the malformations were considered together, no significant dose-response was observed. See Tables 5 and 6 for details.

The NOEL for maternal toxicity was 20.0 mg/kg/day and the fetoembryotoxic NOEL was 60.0 mg/kg. RH-53,866 was not teratogenic at any dose up to 200.0 mg/kg/day, the highest dose level tested.

C. DISCUSSION:

This study was well conducted. However, only 3 litters were available for examination at the highest dose level. Since the EPA testing guidelines call for at least 12 litters to be available for examination at any dose level, this study is classified as CORE MINIMUM.

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ed by: Pamela Hurley PH KD 1/11/88
n 2 , Tox. Branch (TS-76
ury Reviewer: Edwin Budd
n 2 , Tox. Branch (TS-769C)

Budd 1/14/88

006580

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity - In Vitro Cytogenetics (84-2)

TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266099

TEST MATERIAL: RH-3866

SYNONYMS: Rally, Systhane, Myclobutanil

PROJECT NUMBER(S): 20990

SPONSOR: Rohm & Haas Company, Norristown Road, Spring House, PA 19477

TESTING FACILITY: Litton Bionetics, Inc. Kensington, MD 20895

TITLE OF REPORT: Mutagenicity Evaluation of RH-3866 Technical in an In Vitro Cytogenetic Assay Measuring Chromosome Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells

AUTHOR(S): J.L. Ivett and B.C. Myhr

REPORT ISSUED: April, 1985

IDENTIFYING VOLUME: Vol. 25 of 47

CONCLUSION: RH-3866 does not induce chromosomal aberrations either with or without metabolic activation under the conditions of the study.

Classification: ACCEPTABLE

. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-4-chlorophenyl-1H-1,2,4-triazole-1-propanenitrile
Description: amber solid
Batch #(s), Other #(s): Lot # 83159 (Pail #5), TD 84-272
Purity: 91.9%
Source: Rohm & Haas
Vehicle (if applicable): DMSO
Positive Control(s) (if applicable): Mitomycin C, Cyclophosphamide

2. Test Animals and/or Other Test System (if applicable):

Cell Line/Origin: Chinese hamster ovary cells (CHO-WBI)
Source(s): S. Wolff's laboratory, UCSF, California; cloned in A. Bloom's laboratory, Columbia U., New York

3. Protocol:

Cultures were set up 24 hours prior to treatment for both the range-finding test and the aberration assay. The in vitro metabolic activation system consisted of S-9 mix which was derived from Aroclor-induced rats.

Range-Finding Test

For the range-finding test, cultures were exposed to a series of concentrations ranging from 34 ng/ml to 1.02 mg/ml in DMSO. The top dose was limited by the solubility of the test compound.

For the assays without metabolic activation, one day after culture initiation, cells were treated with the test article for 2 hours. Then, 5-bromo-2'-deoxyuridine (BrdU) was added to the cultures and incubation was continued in the dark for approximately 23 hours. The cell monolayers were then washed with saline and complete medium with BrdU. Colcemid was added and the cells were incubated an additional 2.5 hours. The cells were then swollen with KCl hypotonic solution, washed in fixative, dropped onto slides and air-dried.

For the assays with metabolic activation, the cells were incubated at 37°C for 2 hours in the presence of the test article and the S-9 mix. After the 2 hours, the cells were washed with saline and medium containing BrdU. Incubation was continued for a further 23 hours and colcemid was then added. After 2.5 hours, metaphase cells were collected and fixed as described above.

The cell cycle delay was estimated by staining techniques (modified fluorescent-plus-Giemsa, Perry and Wolff, 1974). Slides were stained for 10 minutes with Hoechst 33258 and then mounted and exposed at 55°-60°C to "black light" for the amount of time required for differentiation between chromatids (3-10 minutes). Slides were then stained with 5% Giemsa for 5-20 minutes and air-dried.

Aberration Test

For the assay without metabolic activation, exponentially growing cells were treated with the test article for 17.25 hours. Not all of the specific dosages tested were given. However, the results section states that a range of 25 micrograms/ml through 200 micrograms/ml was tested. The positive control, Mitomycin C was tested at concentrations of 40 ng and 80 ng/ml. The cultures were then washed with saline and colcemid was added with fresh culture medium. Metaphase cells were collected 2 and 1/2 hours later, swollen with KCl hypotonic solution, washed in fixative, dropped onto slides and air-dried. Duplicate cultures were prepared at each dose level.

For the assay with metabolic activation, the cells were incubated at 37°C for 2 hours in the presence of the test article and the S-9 mix. Not all of the specific dosages tested were given but the results section indicates that a range of 20 micrograms/ml through 90 micrograms/ml was tested. The positive control cyclophosphamide was tested at concentrations of 25

and 50 micrograms/ml. The cells were then washed with saline, normal growth medium was added, and they were then incubated for an additional 8.0 hours with colcemid present during the last 2.5 hours of incubation. Metaphase cells were collected fixed, and placed on slides. Duplicate cultures were prepared at each dose level.

Staining and Scoring of Slides

Slides were stained with 5% Giemsa at pH 6.8 for subsequent scoring of chromosome aberration frequencies. One hundred cells were scored from each negative and solvent control and from each of two replicates cultures at four dose levels of the test compound. From the positive controls, 25 to 50 cells were scored from one dose level.

B. RESULTS:

The test article was tested up to the limit of solubility. The maximum dose level tested was 1.02 mg/ml, in which a fine precipitate formed and remained in fine suspension. In the range-finding test without metabolic activation, there was an 88% reduction in monolayer confluency with no dividing cells present at 340 micrograms/ml and 1.02 mg/ml. In the range-finding test with metabolic activation, there was complete toxicity at 102 micrograms/ml through 1.02 mg/ml. The results in Table 1 are reported for dose levels 10.2 micrograms/ml through 102 micrograms/ml. In the test without activation, the test article caused cell cycle delay at 34 micrograms/ml and 102 micrograms/ml. In the test with activation, at 34 micrograms/ml there was a slight reduction in monolayer confluency with no decrease in observable mitotic cells. There was no cell cycle delay. The fixation times were selected for the aberration tests from the results of the range-finding tests.

Tables 2A-3B report the results from the aberration tests. Without metabolic activation, there was complete toxicity at 100 micrograms/ml through 200 micrograms/ml. Results were reported for dose levels 25 micrograms/ml through 75 micrograms/ml. At 75 micrograms/ml, a 38% reduction in monolayer confluency was observed and there were few observable mitotic cells. There was no increase in the percentage of cells with chromosomal aberrations at any dose level scored. With metabolic activation, there was complete toxicity at 60 micrograms/ml through 90 micrograms/ml. At 60 micrograms/ml, there was a 60% reduction in monolayer confluency. Results were reported at 20 micrograms/ml through 50 micrograms/ml. There was no increase in the percentage of cells with chromosomal aberrations at the concentrations analyzed.

In conclusion, under the conditions of the assay, the test chemical did not induce an increase in the percentage of cells with chromosomal aberrations either with or without metabolic activation.

C. DISCUSSION:

This study is ACCEPTABLE. However, there are several points that need to be addressed. First of all, the report was lacking detail. It would have been better if all the dose levels tested had been reported. In addition, in the tables, it would have been useful if the cell cycle progression data had been reported with the aberration studies as it was in the range-finding tests.

This would have allowed the reviewer to determine which cell cycle most of the cells were in for each dose level. Usually this is not necessary in cytogenetic studies after a range finding study and cell cycle analysis. However, in this case it was not entirely clear, for example, how the 17.25 hour incubation time was chosen based on the 25 hour range-finding study without activation. Lastly, for the aberration test with metabolic activation, the fixation time was set at 10 hours. This is somewhat short for a cell culture line that has a dividing time of 12-14 hours. The fixation time could have been longer.

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ed by: Pamela Hurley
n 2 , Tox. Branch (TS-769C)
ary Reviewer: Edwin Budd
n 2 , Tox. Branch (TS-769C)

Budd 1/27/88

006330

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity (Dominant Lethal) - Rats - (84-2)

TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266101

TEST MATERIAL: RH-3866

SYNONYMS: Systhane, Rally, Nova

REPORT NUMBER: 86RC-0054

SPONSOR: Rohm and Haas Company, Philadelphia, PA

TESTING FACILITY: Argus Research Laboratories, Inc., Horsham, PA 19044

TITLE OF REPORT: Dominant Lethal Study of RH-3866 Administered Orally Via Gavage to Crl:COBS®CD®(SD)BR Male Rats

AUTHOR(S): Dearlove, G.E., Hoberman, A.M. and Christian, M.S.

REPORT ISSUED: October 10, 1986

IDENTIFYING VOLUME: Volume 27 of 47, Tab C

CONCLUSION: Technical RH-3866 did not induce dominant lethal mutations in rats up to dose levels of 735 mg/kg.

Classification: UNACCEPTABLE. The study itself was well conducted, but positive control data were lacking. The study will be upgraded to acceptable upon receipt of appropriate historical positive control data from the period this test was performed in the same strain of rats.

. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile
Description: light yellow, crystalline solid
Batch #(s), Other #(s): TD 86-77, Lot #83 159-7
Purity: 91.4%
Source: Rohm and Haas
Vehicle (if applicable): Corn oil

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Charles River Crl:COBS®CD®(SD)BR rats, male and female
Age: 6.5 months (males)
Weight(s): 512-764 grams (males), 194-264 grams (females)
Source(s): Charles River Breeding Laboratories, Inc., Kingston, N.Y.

3. Procedures:

The male rats were assigned to one of the following dose groups (25/group): 0 (vehicle control), 10, 100 or 735 mg/kg of the test substance. The animals were administered the test substance one time via gavage at a volume of 5 ml/kg. The dose levels were selected on the basis of results from an acute toxicity study. Clinical signs of toxicity were observed following a single dose of 689 mg/kg and death was observed following a single dose of 955.8 mg/kg. Following dosing, each animal was assigned two virgin female rats each week for eight consecutive weeks. The females arrived in weekly shipments (50 female rats/group/week). The male rats were examined for general health prior to treatment and observed for any clinical signs of toxicity within 30 minutes after dosage and once daily for the first week postdosage. Thoughout the acclimation and study periods, both males and females were observed twice daily for viability and general appearance. They were also observed for physical signs several times during the acclimation and cohabitation periods. Physical signs for female rats were also recorded on days 0, 6 and 14 of presumed gestation.

Body weights for males were recorded daily during the first week postdosage and weekly bodyweights were recorded thereafter. Body weights of females were recorded on days 0, 6 and 14 of presumed gestation. Body weights of both sexes were recorded at sacrifice. At the completion of the last cohabitation period, each male rat was sacrificed, necropsied, examined for gross lesions, and the testes and epidymides were removed, weighed and preserved in Bouin's solution for possible histopathological examination. During each day of the cohabitation period, each female rat was examined for the presence of spermatozoa in a vaginal smear and/or a vaginal plug in situ (day 0 of presumed gestation). On day 14 of presumed gestation, each female rat was sacrificed, necropsied and the thoracic and abdominal organs were examined for gross lesions. Maternal tissues with gross lesions appropriate for retention were fixed and retained for possible future evaluation. Corpora lutea were identified and counted. The uterus of each rat was examined for pregnancy, number and placement of implantations, early and late resorptions and live and dead embryos (vital status was determined by identification of a beating embryonic heart).

Statistical tests on the data included both parametric and nonparametric tests, depending upon the type of data being analyzed.

B. RESULTS:

One male rat in the highest dose group died two days after administration of the test material. Gross necropsy revealed 3 red-brown, slightly raised areas on the surface of the heart. Clinical signs of toxicity in the highest dose group were observed in a significant ($p<0.01$) number of animals when compared to controls. The signs included chromodacryorrhea, salivation, red oral exudate and dried red perioral material and/or urine stained abdominal fur. Clinical signs related to treatment were not observed in any of the other dose groups.

A statistically significant decrease in body weight gain was observed in the highest dose group during the first two days postdosage. The average body weights for these animals remained lower than the rats in the other groups for the first four weeks of the study. In the weeks thereafter, however, the average body weights were all similar.

There appeared to be no treatment-related lesions at terminal sacrifice. The average testes weights and testes/body weight ratios were similar in all dosage groups. In addition, the number of animals with either one or two small testes in the treated groups were not statistically significant over controls and were similar to numbers observed in historical control animals. Only one animal was infertile (from the 100 mg/kg/day group). The testes of this animal did not appear small at necropsy, but was relatively low in weight. This animal had a lesion in the left inguinal area which was probably a preputial gland abscess.

There were no significant differences in mating performance of the male rats in any of the treated groups. During the first week, however, the average number of matings in the highest dose group was slightly lower than the other dose groups and was attributed to the toxicity of the test material (72.9 % versus 86-88% for the other groups).

No treatment-related differences were observed in the fertility index (average percentage of pregnant rats/number of mated rats).

There were no treatment-related effects observed for any of the parameters used to evaluate dominant lethal mutations. Averages for corpora lutea, implantations, litter sizes (live and dead embryos), resorptions and percentage of dead conceptuses per litter were similar for female rats within all dosage groups in all weeks of mating (see attached histograms). In addition, there were no significant differences in the percentage of female rats/dose group with any resorptions or with all implantations resorbing.

Clinical signs, body weight gains and necropsy findings were similar in all groups of female rats.

DISCUSSION:

This study appears to be well-conducted and excellently presented. However, the study is classified as UNACCEPTABLE because positive control data were lacking. The study will be upgraded to acceptable upon receipt of appropriate historical positive control data from the period this test was performed in the same strain of rats.

ed by: Pamela Hurley
n 2 , Tox. Branch (TS-769C)
ary Reviewer: Edwin Budd
n 2 , Tox. Branch (TS-769C)

Bdd 11/13/88

006580

DATA EVALUATION REPORT

STUDY TYPE: Metabolism (85-1) - Mice TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266102

TEST MATERIAL: RH-3866

SYNONYMS: Rally™, Systhane, Myclobutanil

REPORT NUMBER: 83R-175

SPONSOR: Rohm & Haas Company, Philadelphia

TESTING FACILITY: Rohm & Haas Co., Toxicology Dept., Spring House, PA

TITLE OF REPORT: RH-3866 Kinetic and Metabolism Study in Mice

AUTHOR(S): R.B. Steigerwalt, J.R. Udinsky, S.L. Longacre

REPORT ISSUED: August 29, 1986

IDENTIFYING VOLUME: Vol. 28 of 47

CONCLUSION: Under the conditions of the study, RH-3866 was rapidly absorbed and excreted by the mice. The administered dose was completely eliminated by 96 hours. RH-3866 was extensively metabolized by the animals prior to excretion. The metabolic patterns were similar for both sexes. The results indicated that the disposition and metabolism after oral (pulse) administration in mice were linear over the dose range tested.

Classification: ACCEPTABLE

. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: ^{14}C-alpha-butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazole-1propanenitrile
Batch #(s), Other #(s): Lot 424.0107 (Radiolabeled); Lot LSPL0016E (Nonradiolabeled)
Purity: 81.1% (Nonradiolabeled); 99.7% radiopurity (Radiolabeled)
Specific activity: 10.28 mCi/g (22,822 dpm/microgram)
Source: Rohm & Haas
Vehicle (if applicable): 0.5% methylcellulose

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): male and female Crl:CD-1(ICR)BR mice
Age: 5 weeks
Source(s): Charles River Kingston (Stoneridge, NY)

3. Procedure:

Groups of 3 mice of each sex were fed the following doses of RH-3866 in the diet for 2 weeks: 10, 100, and 1000 ppm. For each dose level, 3 separate groups of 3 males and 3 females were established. The prepared diet was analyzed for homogeneity of mixing and amount active ingredient. Food consumption and body weight gain were measured weekly during the feeding stage of the study. Daily RH-3866 intake was calculated for each animal.

At the end of the 2 weeks, three doses of ^{14}C-RH-3866 were suspended in 0.5% methylcellulose at the following dose levels: 2 mg/kg (2.318×10^6 dpm/ml), 20 mg/kg (4.506×10^7 dpm/ml), and 200 mg/kg (4.229×10^8 dpm/ml). The animals receiving the lowest dietary level received the lowest dose of radioactive chemical, the animals receiving the middle dietary level received the middose of radioactive chemical and the animals receiving the highest dietary level received the highest dose of radioactive chemical. Each animal received a single pulse of the radioactive material orally by gavage at 10 ml/kg body weight.

Blood was collected for ^{14}C-analysis from the first group of animals from the 3 dose levels at 15 and 30 minutes, 1 and 6 hours and at 1 day. These animals were then killed and the carcasses were stored frozen. Excreta (urine and feces) were collected for ^{14}C-analysis and ^{14}C-metabolite profiles from the second group of animals from the 3 dose levels at 6 hours and at 1, 2, 3 and 4 days. These animals were then killed and the carcasses were stored frozen. The third group of animals from the 3 dose levels were killed after 1 hour and the whole blood, plasma and liver were collected for ^{14}C-analysis.

For the metabolite analyses, urine samples were collected at intervals over a 96 hr period from 3 mice at each concentration for each sex and pooled into one sample per sex per dose level. The volume of the pooled sample was adjusted with distilled water and hydrochloric acid and anhydrous sodium sulfate was added. The sample was extracted with tetrahydrofuran (THF) and the organic and aqueous layers were analyzed for total ^{14}C-label. The organic phase was evaporated to dryness and the resulting residue was reconstituted with methanol for thin layer chromatographic (TLC) analysis. Fecal samples were collected over a 96-hour period and homogenized in water. Aliquots of each homogenate were pooled into one sample for the three mice of each sex for each concentration and the metabolites were extracted with the same procedure as the urine samples. The extracts were analyzed with TLC procedures using ethyl acetate: isopropanol:water as the chromatographic solvent. Radioactive areas on the TLC plates were located by autoradiography.

B. RESULTS:

The two-week dietary regimen did not appear to adversely affect the mice as evidenced by the fact that the body weights in males increased by 23-28% and the body weights in females increased by 19-20%. The mean intake of RH-3866 was 2.1, 22.3 and 218 mg a.i./kg/day for mice administered 10, 100 and 1000 ppm (a.i.) RH-3866, respectively. Two animals died due to a gavage error when dosing the animals.

Recovery of ^{14}C-Label

^{14}C-recovery ranged from 80.94 (males; 20 mg/kg) to 107.09% (females; 2 mg/kg). Two females in the 2 mg/kg level had high recovery rates for fecal excretion (110.6 and 194.3%). Consequently, the data from these two animals were not used in the overall recovery calculation.

^{14}C-Excretion

Most of the administered ^{14}C-dose was recovered in the urine and feces within 24-48 hours after dosing. ^{14}C-total excretion by 96 hours post-dose was 60.62-66.62% for males and 66.5-87.15% of the dose for females. An additional 14.09%-23.98% was recovered in the cage washes. Urinary ^{14}C-excretion by 96 hours post-dosing ranged from 20.12-22.20% of the dose for males and 34.95-40.0% of the dose for females. Fecal excretion by the same time period ranged from 38.42-46.5% for males and 30.97-52.2% of the dose for females.

In males, a slightly greater fraction of the excreted ^{14}C-label was eliminated in the feces (0.63-0.70) than in urine (0.30-0.37). The fractions excreted in the urine and feces of the mid- and high-dose females was evenly distributed. The data for the low-dose female group was obtained from only one animal and is thus not reliable.

^{14}C-Whole Blood Concentrations

^{14}C-RH-3866 was rapidly absorbed by the mice. The t 1/2 absorption ranged from 0.04 (2 mg/kg females to 0.31 hrs. (200 mg/kg males). The peak ^{14}C- whole blood concentrations were achieved within 0.25-1 hour. The peak concentrations were 0.36 (2), 6.49 (20), and 34.41 ppm (200 mg/kg) in males and 0.38 (2), 5.26 (20), and 41.88 ppm (200 mg/kg) in females. With the exception of the high-dose males, elimination of the ^{14}C-label from the blood was biphasic. Elimination in the high-dose males showed only 1 phase with a t 1/2 of 6.2 hours. In the other groups, the rapid phase of elimination lasted until approximately 4-6 hours, with half-lives ranging from 0.63 (200 mg/kg females) to 0.88 hours (2 mg/kg females). The half-lives for the slow elimination phase were variable and ranged from 6.0 (200 mg/kg females) to 30.1 hours (2 mg/kg males)

Whole Blood, Plasma, and Liver ^{14}C-Concentrations One Hour After ^{14}C-RH-3866 Dose Administration

For each dose, plasma ^{14}C-concentration was similar to whole blood ^{14}C-concentration for both sexes; liver ^{14}C-concentration was also similar between sexes at each dose. Liver ^{14}C-concentration was 3.9-11.1-fold greater than the corresponding whole blood ^{14}C-concentration. The ^{14}C-label in whole blood at 1 hour post dosing ranged from 1.18 (2 mg/mg females) to 2.03% (20 mg/kg males) of the dose. The percent of label in the liver at 1 hour post dosing was greater than that present in whole blood (2.9-8.4 fold greater). The ^{14}C-label in the liver represented 4.79 (200 mg/kg females) to 10.53% (20 mg/kg males) of the dose. The ratio of the percent of ^{14}C-label in liver to whole blood decreased with increasing dose.

^{14}C-Metabolite Profiles

Urinary ^{14}C-Metabolite Profiles

Up to 15 ^{14}C-fractions were collected from the urine and analyzed, all of these, except one, were more polar than the parent compound. In both sexes, either four (low and mid-dose animals) or five (high dose animals) fractions each contained approximately 10% or more of the urinary ^{14}C-label; these four to five fractions accounted for 57.6 to 70.5% of the urinary ^{14}C-label. A number of other fractions contained approximately 5-10% of the label in either the 20 or 200 mg/kg samples. Some of the fractions in both the 2 mg/kg and 20 mg/kg samples had to be pooled because of the low amount of radiolabel. There were no major differences in the urinary metabolite ^{14}C-profiles between males and females.

Fecal ^{14}C-Metabolite Profiles

As with the urinary metabolites, up to 15 fractions were collected and analyzed and again, all except one were more polar than the parent compound. One fraction in both sexes contained 34.8-46.2% of the fecal ^{14}C-label. Three to four other fractions each contained >10% of the label and five others contained 5-10% of the label. Again, some fractions, particularly in the low-dose animals were pooled and analyzed due to low amounts of radioactive material.

Combined Urinary and Fecal ^{14}C-Metabolite Profiles

There were no major differences in the excreted ^{14}C-metabolite profiles between males and females, nor between the dose levels. One fraction, which co-chromatographed with the parent compound represented 1.1-8.9% of the dose recovered from the excreta (0.7-7.2% of the administered dose).

C. DISCUSSION:

Most of the ^{14}C-label was excreted from the urine and feces within 24-48 hours after dosing. 60-87% of the dose was recovered by 96 hours; 14-24% was recovered from the cage washes at the end of the 96 hours. In males, a slightly greater fraction of the ^{14}C-label was excreted in the feces (0.63-0.70) than in the urine (0.30-0.37). In females, the amount ^{14}C-label excreted was more evenly distributed between urine (0.53) and feces (0.47). The labeled chemical was rapidly absorbed and extensively metabolized. Parent compound excreted was estimated to represent 1-7% of the administered dose. Both sexes produced comparable metabolic profiles at all dose levels. The results indicated that the disposition and metabolism of ^{14}C-RH-3866 after oral (pulse) administration in mice were linear over the dose range studied. This study was used in the selection of doses for the mouse chronic/oncogenicity study. The study is classified as ACCEPTABLE.

ewed by: Pamela Hurley
.on 2 , Tox. Branch (TS-769C)
idary Reviewer: Edwin Budd
.on 2 , Tox. Branch (TS-769C)

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DATA EVALUATION REPORT

STUDY TYPE: Metabolism (85-1) - Rats TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266103

TEST MATERIAL: RH-3866

SYNONYMS: Rally, Systhane, Myclobutanil

REPORT NUMBER: 83R-144

SPONSOR: Rohm & Haas Company, Philadelphia, PA

TESTING FACILITY: Rohm & Haas Company, Toxicology Dept., Spring House, PA

TITLE OF REPORT: RH-3866 Kinetic and Metabolism Study in Rats

AUTHOR(S): R.B. Steigerwalt, J.R. Udinsky, S.L. Longacre and K.L. McCarthy

REPORT ISSUED: August 29, 1986

IDENTIFYING VOLUME: Volume 29 of 47

CONCLUSION: After oral administration, RH-3866 is completely and rapidly absorbed in rats, extensively metabolized, and rapidly and essentially completely excreted. Elimination of the label from the plasma is biphasic and the eliminated dose is evenly divided between urine and feces. No tissue accumulation was observed after 96 hours. Some quantitative differences in metabolites were observed between males and females and pretreatment for 2 weeks with nonlabelled material had little effect on the disposition and metabolism of a single oral dose.

Classification: ACCEPTABLE

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazoyl-1-propanenitrile
Batch #(s), Other #(s): TD83-115, lot 424.0103 (Groups A,C,D); TD83-116, lot 424.0102 (Group B); TD-83-086, lot LSPL0016E (Group D)
Purity: TD83-115: 1.62 mCi/g; TD83-116: 10.28 mCi/g; radiopurity of lot 424.01, of which 424.0103 & 424.0102 were sublots is 99.7%. Nonradiolabeled RH-3866: 81.1% active ingredient
Radiopurity for group A: 97.1%, radiopurity for group B: 97.3%, radiopurity for group C: 96.9%
Source: Rohm & Haas
Vehicle (if applicable): Dimethylisosorbid, methylcellulose

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female Sprague-Dawley Crl:CD®BR rats

Age: 4-6 weeks
Weight(s): 100-175g
Source(s): Charles River Kingston (Stoneridge, NY)

3. Procedure:

The following table from the submitted report (pg. 2) summarizes the experimental design of the study.

Group	Sex	^{14}C-RH-3866 (mg/kg)	Route	n[a]	Time After ^{14}C-RH-3866 Dose Administration: 0.25 hr	0.5 hr	1 hr	6 hr	24 hr	48 hr	72 hr	96 hr
A	M	1	iv	4				E	E	E	E	E,K**
A	F	1	iv	4				E	E	E	E	E,K**
B	M	1	Oral	4				E	E	E	E	E,K
B	F	1	Oral	4				E	E	E	E	E,K
C	M	100	Oral	2	P		K					
C	M	100	Oral	2			P	K				
C	M	100	Oral	2		P		P	K			
C	M	100	Oral	2		P			P	K		
[illegible]	M	100	Oral	4				E	E	E	E	E,K
[illegible]	F	100	Oral	4				E	E	E	E	E,K
D	M	100[b]	Pulse	2	P		K					
D	M	100[b]	Pulse	2			P	K				
D	M	100[b]	Pulse	2		P		P	K			
D	M	100[b]	Pulse	2		P			P	K		
D	M	100[b]	Pulse	4				E	E	E	E	E,K
D	F	100[b]	Pulse	4				E	E	E	E	E,K

n: number of animals

These rats received a single dose of 100 mg/kg ^{14}C-RH-3866 following 14 day dietary exposure to 1000 ppm (ai) nonradiolabeled RH-3866 (81.1% ai).

Excreta (urine, urine funnel washes, and feces) were collected for ^{14}C-analysis. For Groups B, C, and D, urine and fecal samples containing the majority of the excreted ^{14}C-label were each pooled for analysis of ^{14}C-metabolite profiles.

Whole blood and plasma were collected for ^{14}C-analysis.

Rats were killed and whole blood, plasma, liver, kidney, lung, heart, spleen, brain, adrenals, thyroid, fat, bone marrow, testes (males), uteri/ovaries (females), and muscle were collected and analyzed for ^{14}C-label.

[illegible]: Rats were killed and carcasses were stored frozen.

The intravenous doses of ^{14}C-RH-3866 were administered at 1 ml/kg body weight into an exposed femoral vein. Oral doses were administered by gavage at 10 ml/kg body weight. Nonradiolabeled RH-3866 was administered in the diet ad lib. to the animals in group D. These animals were returned to untreated diet upon administration of the pulse (oral) dose of ^{14}C-RH-3866. Samples of the diet were assessed for homogeneity and concentration of test substance. Dietary and RH-3866 intake for each animal were calculated from the weekly feed consumption and body weight values. The amount of ^{14}C-RH-3866 dosed per animal was also calculated from the volume of the ^{14}C-dose solution/suspension administered and the liquid scintillation counting (LSC) analysis of the ^{14}C-dosing solution/suspension.

Individual animal weights were recorded when the animals were randomized, at the initiation of the dietary feeding stage, prior to ^{14}C-dose administration and at termination. Aliquots of urine were analyzed for total ^{14}C-label by LSC and the remaining samples were stored frozen until analysis for ^{14}C-metabolites. Feces were collected and stored frozen until analysis (quantitation of ^{14}C-label and ^{14}C-metabolites). Blood was collected by orbital sinus puncture. The percent of dose in whole blood was calculated assuming that whole blood represented 6.5% of a rat's body weight.

The organ samples were removed, weighed and analyzed for ^{14}C-label by combustion and LSC. The percent of dose in bone marrow, fat and muscle was calculated assuming these tissues represented 0.4, 7.0 and 45.5% of body weight, respectively.

Cage washes and urine collecting funnel washes were kept separate and analyzed by LSC. Where appropriate, the results were combined with those obtained from direct measurements of body fluids and/or tissues.

Pharmacokinetic Parameters

The ^{14}C-plasma and ^{14}C-whole blood data for high (100 mg/kg; Group C) and pulse dosed male rats (100 mg/kg; Group D) were each fitted to an equation describing an open two-compartment model. For other tissues, linear regression analysis was performed on all time course data prior to half-life determinations.

Determination of Percent Oral Dose Absorbed

The percent oral dose absorbed was determined by the relative percent of the dose excreted in the urine and urine funnel wash of orally dosed rats divided by the relative percent of dose excreted in the urine and urine funnel wash of i.v. dosed rats.

Metabolite Analysis:

Urine samples were pooled for individual rats. The samples were treated with hydrochloric acid and anhydrous sodium sulfate and were then extracted with tetrahydrofuran (THF). Both the organic and aqueous fractions of each sample were analyzed for total ^{14}C-label to determine extraction efficiency. The extracted samples were analyzed by thin layer chromatography (TLC). Fecal samples were also pooled for individual rats. The samples were extracted with methanol and analyzed by TLC.

The solvent system used for the TLC plates consisted of ethyl acetate, isopropanol and water. The radioactive areas on the TLC plates were located by autoradiography and quantitated by scraping the spots from the plates and analyzing by LSC. R_f values were determined for each fraction.

B. RESULTS:

Body weights were not affected by treatment. Animals that were on test for the entire 96 hr. in-life phase showed a 4-27% increase in body weight. Animals that received RH-3866 in the diet for two weeks showed a mean 23 and 12% increase in body weights for males and females, respectively. Mean RH-3866 intake was calculated to be 83-84 mg a.i./kg/day.

Recovery of ^{14}C-Label

For the animals dosed intravenously, the recovery value was based on ^{14}C-label recovered from urine, urine funnel washes, feces, and cage washes. The ^{14}C-label recovered from the skin and remaining carcass from one animal was also used in the determination. For orally dosed animals, the total recovery of ^{14}C-label was determined from the urine, urine funnel washes, feces, cage washes and tissues.

The ^{14}C-recovery from ^{14}C-RH-3866 iv dosed animals was 77.43 and 81.98% for males and females, respectively. The calculated values did not include the results from one male and two females which showed low recovery values. The authors believe that these low values were due to misdosing. The ^{14}C-recovery values from ^{14}C-RH-3866 orally dosed animals ranged from 82.35 to 96.78%.

^{14}C-Excretion

In iv dosed rats, 54.86-64.36% of the dose (71-79% of the recovered ^{14}C-label) was found in the urine and feces within 24 hours post dosing. Total ^{14}C-excretion within 96 hours post-dosing was 72.60% (males) and 78.72% (females), which represented 94-96% of the recovered C^{14}-label. The excreted ^{14}C-label was essentially evenly distributed between the urine and feces (35.34-41.01% and 31.59-43.39%, respectively). In males, the fraction of the excreted dose eliminated in the urine and feces was 0.56 and 0.44, respectively, and in females, the fraction of the excreted dose eliminated in the urine and feces was 0.45 and 0.55, respectively.

In orally dosed rats, 40.80-81.11% of the dose (males and females, 55-93% of the recovered ^{14}Clabel) was found in the urine and feces within 24-48 hours after dosing. Total ^{14}C-excretion by 96 hours post dosing ranged from 73.91 to 88.12% of the dose, which represented 89-98% of the recovered ^{14}C-label. The

excreted ^{14}C-label was evenly divided between the urine and the feces; the fraction of the excreted dose eliminated in the urine and feces was 0.45-0.57 and 0.43-0.55, respectively. Most of the ^{14}C-urinary and fecal excretion was completed within 24 hours after dosing. By 96 hours, ^{14}C-urinary excretion ranged from 36.60%-48.43% of the dose. ^{14}C-fecal excretion by 96 hours ranged from 34.27%-45.56% of the dose.

Percent of Dose Absorbed After Oral Administration

89.2-114.6% of the dose of ^{14}C-RH-3866 was absorbed by rats following oral administration of a low (1 mg/kg), high (100 mg/kg) or pulse (100 mg/kg) dose of ^{14}C-RH-3866.

^{14}C-Plasma and ^{14}C-Whole Blood Concentrations

^{14}C-plasma and ^{14}-whole blood data from male rats administered a single oral or a pulse oral dose of 100 mg/kg ^{14}C-RH-3866 were fitted to equations describing an open two compartment model. In the plasma, ^{14}C-label was rapidly absorbed after administration of 100 mg/kg ^{14}C-RH-3866 [t 1/2 absorption = 0.21 (group C) and 0.24 hr (Group D)], achieving peak ^{14}C-plasma concentrations of 19.56 (Group C) and 23.75 ppm (Group D) within 1 hour after dosing. The rapid phase of elimination for single oral dosed male rats lasted until approximately 24 hours postdose (t 1/2 rapid phase = 5.25 hr); the rapid phase of elimination for pulse dosed male rats lasted until approximately 12 hours post-dose (t 1/2 rapid phase = 1.97 hours). The slow elimination phases of single oral and pulse dosed rats were comparable (t 1/2 slow phase = 25.67 to 31.50 hours, groups C and D, single and pulse dosed rats, respectively).

The ^{14}C-whole blood concentration time curves for single oral and pulse oral dosed rats were very similar to the ^{14}C-plasma concentration time curves. There were several differences: a) the rapid phase of elimination in single orally dosed rats was faster and shorter in duration in whole blood than in plasma and b) the slow elimination phase was slightly slower and the area under the ^{14}C-concentration time curve was slightly greater in whole blood than in plasma for both single and pulse orally dosed rats.

Tissue ^{14}C-Concentration

^{14}C-label rapidly appeared in all tissues, reaching peak concentrations by 1 hr post-dose for all tissues except liver of single dosed male rats, where the peak ^{14}C-concentrations observed at 6 hours post-dose was only 9% greater than the ^{14}C-concentration observed at 1 hour post-dose. Peak 1 hour ^{14}C-concentrations ranged from 6.29 (brain) to 56.65 ppm (liver) for single dosed male rats and ranged from 15.01 (brain) to 153.5 ppm (liver) for pulse dosed male rats. The peak tissue to whole blood ratio 1 hour after dosing ranged from 0.2 (brain) to 2.2 (liver) for single dosed male rats, and ranged from 0.8 (brain) to 7.7 (liver) for pulse dosed male rats. The peak (1 hour) tissue ^{14}C-concentrations

120

of pulse dosed male rats were 0.8- (whole blood) to 8.1-fold (spleen) greater than the corresponding peak tissue ^{14}C-concentrations of single dosed male rats. For each tissue (except whole blood), the peak (1 hr) ^{14}C-concentration and the 1 hour tissue/blood ratio were greater in the pulse dosed male rats than in single dosed male rats.

Elimination of the label from the tissue was similar to that of the plasma and blood, i.e. biphasic. The rapid elimination phase occurred from approximately 1-24 hours post dosing. This was followed by a slower elimination phase. In single dosed male rats, the t 1/2 for the rapid phase ranged from 1.61 (whole blood) to 11.50 hours (liver) and the t 1/2 for the slow phase ranged from 25.67 (plasma) to 51.31 hours (thyroid). Elimination from the adrenals was monophasic: t 1/2 = 16.34 hours. In pulse dosed male rats, the t 1/2 for the rapid phase ranged from 1.97 (plasma) to 8.26 hours (adrenals), and the t 1/2 for the slow phase ranged from 29.73 (rat) to 143.0 hours (kidney). For each tissue except whole blood and brain, the rapid phase of elimination was slightly faster and the slow phase of elimination was slightly slower for pulse dosed male rats than for single dosed male rats.

Tissue ^{14}C-Concentrations 96 Hours Post-Dose

The amount of ^{14}C-label remaining in the tissues of low-dosed rats (single oral dose) 96 hours post dosing was 0.73 and 0.40% of the dose for males and females, respectively. Liver and kidney ^{14}C-concentrations in males were 2.7 to 4.0-fold greater than the respective tissue ^{14}C-concentrations in females. The amount of ^{14}C-label remaining in the tissues of high-dosed rats (single oral dose) 96 hours post dosing was 0.31 and 0.06% of the dose and males and females, respectively. No significant accumulation of ^{14}C-label in tissues was observed. ^{14}C-concentrations in blood, liver, kidney, spleen, heart, lung and muscle of males were 4.8-8.8-fold greater than the respective tissue ^{14}C-concentrations in females at 96 hours post-dose.

In pulse dosed rats, ^{14}C-concentrations in blood, liver, kidney, spleen, heart, lung and muscle of males were 3.5- to 7.0-fold greater than the respective tissue ^{14}C-concentrations in females at 96 hours post dose. For each sex, the respective tissue ^{14}C-concentrations at 96 hour post-dose were comparable between 100 mg/kg single and pulse dosed rats. The amount of ^{14}C-label remaining in the tissues of pulse dosed rats (100 mg/kg) was 0.53 and 0.08% of dose for males and females, respectively.

^{14}C-Metabolite Profiles

The ^{14}C-metabolite fractions from pooled urine and pooled fecal samples from each dose group were separated by thin layer chromatography (TLC), located by autoradiography, scraped from the plates and counted by liquid scintillation counting.

Urinary ^{14}C-Metabolite Profiles

Extraction of ^{14}C-label from the urine was quantitative (96.6-98.6%). Fifteen fractions were collected; 13 were more polar than the parent compound. In males, 5 fractions each contained 10% or more of the urinary ^{14}C-label, accounting for 68.3-78.7% of the label. The amount of each of these fractions was similar in all tested groups. Four fractions contained approximately 5-10% of the urinary ^{14}C-label. In females, 2 fractions contained 54.5-69.2% of the urinary ^{14}C-label. Pulse-dosed females excreted a lesser amount of the major urinary metabolite than single-dosed females (5.6% versus 13.8-19.0%); and low dose females excreted a lesser amount of the second major metabolite than either high or pulse-dosed females (4.4% versus 10.1% and 11.9%). However, it should be noted that only one adequate urinary ^{14}C-metabolite profile was obtained for analysis from low-dosed females. Four to five fractions in the females each contained 5-10% of the urinary ^{14}C-label.

Fecal ^{14}C-Metabolite Profiles

In several instances, the fecal fractions had to be combined for analysis. All ^{14}C-metabolite fractions except one were more polar than the parent compound. In males, 5 fractions contained 10% or more of the ^{14}C-label. These five fractions accounted for 75.1 to 81.9% of the fecal ^{14}C-label. There was no major difference in the amount of the fractions excreted in the feces among the low, high and pulse dosed rats. Males also excreted 3 fractions in the feces that each contained approximately 5-10% of the fecal ^{14}C-label. In females, one fraction was dominant in the feces, containing 52.5% (low dose) and 77.2-79.8% (high and pulse) of the fecal ^{14}C-label. In addition, five fractions were excreted that each contained 5-10% of the fecal ^{14}C-label.

Combined Fecal and Urinary Profiles

In males, 5 fractions each contained approximately 10% or more of the ^{14}C-label. These 5 fractions accounted for 60.6-78.8% of the excreted ^{14}C-label. For each fraction, there was no major difference in the amount excreted for any of the animal groups analyzed. The males also eliminated 3 fractions in the excreta that each contained approximately 5-10% of the excreted label. In females, one fraction was dominant. This fraction contained 53.3-61.1% of the excreted ^{14}C-label. One other fraction was excreted that contained greater than 10% of the excreted label and 7 fractions contained between 5-10% of the excreted label. The female excretion pattern was slightly different from the male excretion pattern.

C. DISCUSSION:

The results indicated that the disposition and metabolism of the chemical following oral administration of either 1 mg/kg or 100 mg/kg was similar. The amount of ^{14}C-labelled metabolites excreted in feces and urine was similar for both groups (nearly evenly distributed between urine and feces), the concentration of ^{14}C-label in tissues 96 hours after the ^{14}C-RH-3866 dose administration was essentially proportional to dose and the ^{14}C-metabolite profiles were qualitatively and quantitatively similar between the two groups. Some sex differences were noted. The concentration of ^{14}C-label in blood, liver, kidney and a number of tissues 96 hour post dosing was greater in males than in females, and females excreted a greater amount of one fraction and lesser amounts of several other fractions than the males. The results indicated that for the most part, pretreatment for two weeks with the test chemical in the diet had little effect on the metabolism and distribution of a pulse dose of the test chemical when compared to results from animals that were not pretreated. The differences observed included: a) peak tissue concentrations (except plasma and whole blood) were greater in pretreated rats than in nontreated rats and b) the rapid phase of elimination in most tissues was slightly faster in pretreated rats and the slow phase of elimination in pretreated rats was slightly slower in pretreated rats. The authors of the report speculate that this may have been partly due to the fact that the chemical has been shown to induce hepatic mixed-function oxidase activity in rats in subchronic and chronic studies.

The results of the study also indicated that practically the entire oral dose of the chemical was rapidly absorbed in rats and that the chemical was extensively metabolized. The parent compound was estimated to represent 1.0-3.6% of the excreted dose. Following a single i.v. dose, 71-79% of the recovered ^{14}C-label of the dose was eliminated in the excreta within 24 hours and 94-96% of the recovered ^{14}C-label was eliminated in the excreta within 96 hours. Following a single oral dose, the amounts were 55-93% within 24-48 hours and 89-98% within 96 hrs.

The authors stated that results from a previous paper indicated that RH-3866 is not readily biotransformed to volatile metabolites such as carbon dioxide. Approximately 0.01-0.02% of the recovered label from a single oral dose was obtained from the expired breath of the rats tested.

The study appears to have been thoroughly conducted. The following are a few comments concerning the study. First, there was no indication of the purity of the active ingredient in the radioactive samples. It is assumed that these were synthesized in the same manner as the nonradioactive material. This needs to be clarified. Second, the identity of the metabolites is dependent upon comparison of this study with a distribution and metabolism study conducted in 1984. The comparison shows some distinct similarities in the metabolites produced by the rats in the two studies. Some of the percentages of metabolites varied and some of the R_f values on the TLC plates varied, but the variations were consistent and thus, the Toxicology Branch believes that the two studies can be used in combination with each other to provide acceptable and complete distribution and metabolism data. The study is ACCEPTABLE.

123

ed by: Pamela Hurley
1 2 , Tox. Branch (TS-769C)
ary Reviewer: Edwin Budd
1 2, Tox. Branch (TS-769C)

006580

Budd 11/13/88

DATA EVALUATION REPORT

STUDY TYPE: Metabolism (rats) 85-1 TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 072904

TEST MATERIAL: RH-3866

REPORT NUMBER: 310-84-16

SPONSOR: Rohm & Haas Company, Philadelphia, PA

TESTING FACILITY: Spring House Research Laboratories

TITLE OF REPORT: A Material Balance and Metabolism Study of ^{14}C RH-3866 in Rats

AUTHOR(S): D.R. Streelman

REPORT ISSUED: June 22, 1984

CONCLUSION: RH-3866 is extensively metabolized and excreted in the urine and feces. At least 7 major metabolites were recovered and specifically identified. Recovery of the radioactivity administered averaged at 97.2%. The highest amounts of radioactivity found in the body were in excretory organs: liver, kidneys, and large and small intestines.

Classification: Acceptable when combined with other rat metabolism study.

. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile
Description: glassy compound
Batch #(s), Other #(s): lot # 417.01
Purity: Not given
Source: Rohm and Haas
Vehicle (if applicable): 0.5% methyl cellulose in water

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female Sprague-Dawley rats
Age: Not given
Weight(s): Approximately 150 gm.
Source(s): Not given

3. Procedures:

Four male and four female rats were housed in metabolism cages. Each animal was given a single dose by gavage of 2000 ppm ^{14}C-RH-3866 in 1 ml dosing solution (approximately 150 mg/kg bodyweight). Urine and feces were collected and CO_2 was trapped for six hours, 24 hours after dosing and each day thereafter until sacrifice. The carbon dioxide trapping solutions, urine and feces were stored frozen until used. Two animals of each sex were sacrificed four days after dosing and the remaining animals were sacrificed seven days after dosing. The following tissues were removed and frozen until analysis:

Adrenal
Bladder
Blood
Bone
Brain
Fat
Gonads
Heart
Kidney
Lg. Intestine
Liver
Lungs
Muscle
Pituitary
Skin/Hair
Spleen
Small Intestine
Stomach
Thymus
Thyroid
Carcass

Radioassay of organs and tissues were carried out on only one animal of each sex at each sacrifice time. Urine and CO_2 samples were counted directly. Feces and tissue samples were homogenized, combusted and counted. Urine and feces were assayed for presence of metabolites using thin layer chromatography and radioautography.

The metabolites were separated and characterized. The ethyl acetate fractions from the preliminary purification were further purified on TLC plates useing the following solvent system: ethyl acetate - isopropanol - water (85:13:2). The butanol fraction from the female feces extract were further purified by TLC using the same solvent system but with the following ratios: ethyl acetate - isopropanol - water (65:25:10). Compounds 1-4 and 6 from the preceding isolation procedure were analyzed by gas chromatography - mass spectroscopy and compound 5 was characterized by fast atom bombardment MS (FAB/MS) and direct insertion probe MS. Compound 7 was studied with FAB/MS and ^{1}H and ^{14}C nuclear magnetic resonance spectroscopy.

Urine and feces from the first four days after dosing were analyzed by TLC to quantitate the distribution of metabolites. The same solvent system was used as above with the following ratio: 65:25:10. Standards of the isolated metabolites were spotted along with the urine samples. The plates were autoradiographed and radioactive zones were marked. These areas were scraped from the plates and counted.

RESULTS:

Excretion Pattern

Most of the radioactivity was excreted in the urine and feces. Very little was excreted in the expired CO_2. An average of 99.3% of the recovered radioactivity was contained in the urine and feces, excluding cage washes. The average overall recovery of radioactivity was 97.2%. The distribution of radioactivity between the urine and feces varied considerably from animal to animal. The excretion pattern fit a first order kinetic model. The half life of clearance in the female was 11 hours and the half life of clearance in the male was 15 hours.

Tissue and Organ Levels of ^{14}C

The highest concentrations of radioactivity were found in the excretory organs: liver, kidney, and small and large intestines. There was no apparent accumulation in any organs or tissues. The residues were lower in the female than they were in the male.

Metabolite Identification

The metabolites were all more polar than the parent compound. The same metabolites were present in both urine and feces and in both sexes but in different proportions. Figure I and Table I give the structures of the metabolites recovered and the proportion in which they were found for each sex.

DISCUSSION:

This study appears to be well conducted. When combined with the other rat study conducted on this pesticide (1986), it is acceptable as having fulfilled the requirements for metabolism studies on the chemical. When the data obtained from these two metabolism studies were compared with the metabolism data on plants, cows and chickens, the Toxicology Branch decided that no further toxicity studies on the metabolites were required because the rat metabolizes RH-3866 to significant amounts of the metabolites that were recovered from plants, cows and chicken eggs. Therefore, in the toxicity studies on the parent compound, the rat was also exposed to significant amounts of the metabolites of interest as well.

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

July 15, 1987

SUBJECT: RH-3866, Review of Dermal Absorption Study

TO: Pam Hurley PhD
Pharmacologist
Toxicology BRanch

FROM: Robert Poizendzian PhD
Senior Pharmacologist
Toxicology Branch

Action Requested

Review the following study;

RH-3866 Dermal Absorption study in male rats, Protocol No 85P-394, L.J. DiDonato & R.B. Steigerwalt, Rohm and Haas Co, Report No. 85R-179, Aug 26, 1986. Accession #266104.

Conclusions

Failure to perform analysis of application site skin and residue in the carcass make it impossible to varify recovary. The study is unacceptable.

Recomendation

It is recomended that the Registrant analyse application site skin and the carcasses of the animals used in the study in order to complete the material balance determination. Submission of this data can make the study acceptable.

Discussion

This study follows the general pattern of acceptable dermal absorption studies but it fails in that the Laboratory utilizes the "Maibach" correction in place of analyzing the skin at the application site and carcasses. The "Maibach" correction is designed for studies in which one cannot sacrifice the experimental animals for determination of residue in the body. The correction utilizes an intravenous dose in the experimental animal to generate a correction factor for excretion of absorbed compound. This approach has never been experimentally proven. It generally fails by using the same dose intravenously as was used dermally. In this case the iv

dose is 1/1347 of the absorbed high dermal dose and 1.8 times the absorbed low dermal dose. Also an intravenously administered dose is a systemic pulse dose while a dermal dose slowly enters tne body.

Considering that 107.95 and 112.12 percent of the resepective dermal doses were accounted for, application of the correction factor in this study is 'redundent'.

The Registrant applied the correction factor solely to urinary excretion and failed to include fecal excretion thereby underestimating dermal absorption by a factor of approximately two.

Also it must be noted that considerably less effort is required to analyze eight pieces of rat skin and eight rat carcasses than to handle the entire intravenous dose group.

006580

Data Evaluation Report

Compound RH-3866 [2-butyl-2 (4-chlorophenyl)-1H-1,2,4-triazolyl-1-propanenitrile]

Citation

RH-3866 Dermal Absorption study in male rats, Protocol No 85P-394, L.J. DiDonato & R.B. Steigerwalt, Rohm and Haas Co, Report No. 85R-179, Aug 26, 1986. Accession #266104.

Reviewed by Robert P. Zendzian PhD 7/1/87
Pharmacologist

Core clasification Unacceptable

Conclusions

Failure to perform analysis of application site skin and residue in the carcass make it impossible to varify recovary.

Materials

1. ^{14}C-RH-3866 [2-butyl-2 (4-chlorophenyl)-1H-1,2,4-triazolyl-1-propanenitrile] TD85-231; Lot 424.0126; 10.26 uCu/mg (22,777 dpm/ug).

2. ^{14}C-RH-3866; TD85-234; lot 424.012a; 1.026 uCi/mg; (2,278 dpm/ug. Note: TD85-234 was composed of 10.5 mg ^{14}C-RH-3866 (lot 424.0126, 10.26 uCi/mg; light amber oil) plus 94.5 mg of nonradiolabeled RH-3866 (lot RP08154-5; >99% ai).

3. 2EC Formulation blank (Vehicle): TD85-232; Lot EG1097; brown tinged liquid.

Male Sprague-Dawley Crl:CD®BR rats; Charles River Breeding Laboratories.

Experimental Design

Four Rats per group were randomly assigned to each treatment group. Dermal doses were applied in 60 ul on a 2 x 2 cm area. RH-3866 2EC contained 250 mg ai/ml. The dose for the intravenous group was dissolved in DMSO.

Dosing and sample collection were according to the following table from the report;

006580

Gp	Route	RH-0265 2EC Dilution	Dose ^{14}C-RH-3866 (ug/cm^2)	(mg/kg)	(ug/rat)	Time after ^{14}C dose 6hr	1d	2d	3d	4d	5d	6d	7d
	Dermal	None	3,750	77±7	15,000	W,E	E	E	E	E	E	E	E,K
	Dermal	1:400	9.4	0.20±0.02	37.5	W,E	E	E	E	E	E	E	E,K
	IV	—	———	0.16±0.02	30	E	E	E	E	E	E	E	E,K*

The application sites were wipec with soap and water and analyzed for ^{14}C-label.
Urine, feces and urine funnel washes were collected for ^{14}C-analysis.
Animals were killed, the application site skins removed, and stored frozen along with the remaining carcasses.
Animals were killed and carcasses were stored frozen.

"The back (dorsal lumbar regicn) of each animal was shaved the day before ^{14}C-dose application. Immediately prior to dose application, a 2 x 2 cm square was drawn within the shaved region on each animal's back, and a contoured glass ring (3.6 cm diameter, 10.2 cm^2) was secured around the drawn square on each animal's back with cyanoacrylate type adhesive. Each glass ring waas equipped with a porous top. The ^{14}C-dose was applied in a 60 ul volume within the 2 x2 cm area using an Eppendorf Microliter Pipette (Brinkman Inc.)."

The following samples were collected and analyzed for ^{14}C;
1. Soap and water wash solutions
2. Urine and feces
3. Funnel wash
4. Application site skins frozen not analyzed
5. Remaining carcass stored frozen not analyzed
6. Entire cage wash

Results

Results are summerized in the Table 1.

Discussion

This study follows the general pattern of acceptable dermal absorption studies but it fails in that the Laboratory utilizes the "Maibach" correction in place of analyzing the skin at the application site and carcasses. The "Maibach" correction is designed for studies in which one cannot sacrifice the experimental animals for determination of residue in the body. The correction utilizes an intravenous dose in the experimental animal to generate a correction factor for excretion of absorbed compound. This approach has never been

experimentally proven. It generally fails by using the same dose intravenously as was used dermally. In this case the iv dose is 1/1347 of the absorbed high dermal dose and 1.8 times the absorbed low dermal dose. Also an intravenously administered dose is a systemic pulse dose while a dermal dose slowly enters the body.

Considering that 107.95 and 112.12 percent of the resepective dermal doses were accounted for, application of the correction factor in this study is 'redundent'.

The Registrant applied the correction factor solely to urinary excretion and failed to include fecal excretion thereby underestimating dermal absorption by a factor of approximately two.

Also it must be noted that considerably less effort is required to analyze eight pieces of rat skin and eight rat carcasses than to handle the entire intravenous dose group.

Table 1. Mean recovary of ^{14}C label as percent of applied dose. Data from Table III of the report.

Group	RH-3866 2EC Dilution	Dose ^{14}C-RH-3866 (ug/cm^2)	(mg/kg)	(ug/rat)	Urine	Feces	Funnel wash	Cage wash	Ring wash	Wipes	Total Absorbed[a]	Total recovary
A der	None	3,750	77±7	15,000	10.13	12.93	2.43	1.45	1.88	79.14	26.94	107.95
B der	1:100	9.4	0.20±0.02	37.5	17.65	19.90	3.29	3.33	3.56	64.38	44.4	112.12
C iv	---	-----	0.16±0.02	30	57.75	47.73	11.45	6.73				123.66

a. Total of urine, feces, funnel wash and cage wash.

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CONFIDENTIAL APPENDIX

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