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DATA EVALUATION REPORT
RH-893 HQ Technical

Study Type: Subchronic Dermal Toxicity: 90-Day Study

Study Title: RH-893 HQ Technical Three-Month Dermal Toxicity Study in Rats

Prepared for:

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Guideline 82-3
90-Day Subchronic Dermal Toxicity Study

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DATA EVALUATION REPORT

STUDY TYPE: Subchronic Dermal Toxicity: 90-Day Study

TEST MATERIAL: RH-893 HQ technical

TOX CHEM. NUMBER: 613C

SYNONYMS: Octhilinone

PC NUMBER: 099901

STUDY NUMBER: 90R-031

MRID NUMBER: 420073-01

SPONSOR: Rohm and Haas Company

TESTING FACILITY: Rohm and Haas Company, Toxicology Department, 727 Norristown Road, Spring House, Pennsylvania 19477

TITLE OF REPORT: RH-893 HQ Technical Three-Month Dermal Toxicity Study in Rats

AUTHORS: Bernacki HJ and Hamilton JD

REPORT ISSUED: 08/19/1991

CONCLUSIONS: NOEL (dermal) <2.97 (0.3%) mg a.i./kg/day
LEL (dermal) = 2.97 (0.3%) mg a.i./kg/day (skin irritation includes hyperkeratosis, acanthosis, foci of necrosis, eschar formation, sebaceous gland hyperplasia and chronic inflammation)

NOEL (systemic) = 5.95 (0.6%) mg a.i./kg/day
LEL (systemic) = 14.87 (1.5%) mg a.i./kg/day (based on decreases in HGB, HCT, RBC, albumin, glucose and total protein in females and a decrease in body weight and body weight gain in males.

CORE CLASSIFICATION: Core Minimum. This study is classified as Core Minimum because the test site was not occluded after dose administration, and data for the differential leukocyte count were not provided.

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A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: RH-893 HQ Technical

Active ingredient: Technical grade contains 99.1% of 2-n-octyl-4-isothiazolin-3-one

Vehicle: Propylene glycol

Identification: Toxicology department sample no. 90-009; batch no. 3192

Source: Rohm and Haas Company

Purity: 99.1%

Physical description: Yellow amber liquid

2. Test Article Analyses for Concentration and Stability

Solutions were prepared once for the entire study prior to the initiation of dosing and were submitted for determination of concentration and stability. Triplicate samples were submitted at weeks 0, 4, 8, and 13 to determine stability in propylene glycol at 0.02% and 2.0% w/w a.i. Triplicate samples were submitted on days 1, 34, and 65 of treatment to verify concentrations of 0.3%, 0.6%, and 1.5%.

Stability ranged from 89% to 122% of target with a standard deviation of 13. Concentrations of the test substance in the dosing solutions ranged from 95% to 110% of target concentrations for all dose groups, with a standard deviation of 5.

3. Test Animals

Species: Rat

Strain: Crl:CDBR

Source: Charles River Breeding Laboratories, Inc. Kingston Facility (Stone Ridge, NY)

Sex: Males and females

Age at study start: Males, 6 weeks; females, 6 weeks

Weight at study start: Males, 224 grams; females, 164 grams

The animals were acclimated to the study room for approximately 2 weeks prior to the initiation of treatment. All animals were individually housed in stainless steel cages. Animals were uniquely identified by a number tattooed on their tails. All animal rooms were environmentally controlled, with controls set to maintain a temperature of 73°F and a relative humidity of 40-60%. Temperature and relative humidity were monitored 24 hours a day. The light cycle was on automatic control with 12 hours of light and 12 hours of dark. All rats were fed Certified Purina Laboratory Rat Chow #5002 ad libitum. Tap water was available ad libitum through an automatic watering system.

The animals were randomly assigned to one of the five study groups based on a computerized distribution according to body weight. Each group consisted of 10 female and 10 male rats as stated in Guideline 82-3 requirements.

Dose Preparation and Dose Administration: See Table 1 for a description of the experimental design describing treatment groups for this study.

An untreated control group and a vehicle control group that received daily 1-mL/kg applications of propylene glycol were used for this study. Three treatment groups received daily 1-mL/kg applications of RH 893 HQ technical at doses of 2.97, 5.95, or 14.87 mg a.i./kg-day. The doses used in this study were selected based on results found in a preliminary range-finding study. Summaries of the dose-finding study design, results, and dose selection rationale were included in the report.

RH-893 HQ technical was prepared as a weight-to-weight solution in propylene glycol and administered topically (non-occluded) to Crl:CDBR rats (10/sex/group), 5 days a week, over a 13-week period resulting in a total of 65-67 daily applications. This study deviated from the Guideline (82-3) by not applying an occlusive dressing after dose administration; the authors stated that the test site was non-occluded. Guideline 82-3 states that the test substance is to be held in contact with the skin with a porous gauze dressing and non-irritating tape. Also, the test site should be further covered in a suitable manner to retain the gauze dressing and test substance and to ensure that the animals cannot ingest the test substance. However, the authors did state that the animals were fitted with cardboard collars beginning 1 week prior to dosing and throughout the dosing

TABLE 1. Experimental Design of Three-Month Dermal Toxicity Study in Rats of RH-893 HQ Technical

Group	Treatment	Percent (%)	Daily Dose (mg a.i./kg)[a]	(mL/kg)	No. of Rats/Sex
1	Control (untreated)	0.0	0.00	0	10
2	Vehicle control[b]	0.0	0.00	1	10
3	RH-893 HQ in vehicle	0.3	2.97	1	10
4	RH-893 HQ in vehicle	0.6	5.95	1	10
5	RH-893 HQ in vehicle	1.5	14.87	1	10

[a]a.i.: active ingredient (2-n-octyl-4-isothiazolin-3-one) of RH893 HQ Technical
[b]vehicle: propylene glycol

period. On the day prior to the first treatment, the hair around the entire trunk between the flank and shoulders of animals in each study group was closely shaved with an electric shaver. A 4 x 5 cm area was designated on the back of each rat to ensure dosing of the same area. The use of a 4 x 5 cm area satisfied the Guideline requirement that an area not less than approximately 10% of the total body surface area should be cleared for test substance application. The shaving procedure was repeated as needed during the study.

4. Statistical Methods

The statistical methods used for analysis of continuous data (body weight, body weight gain, cumulative body weight gain, feed consumption, feed efficiency, organ weight, relative organ weight, clinical chemistry, and hematology, except WBC differential) included Analysis of Variance (ANOVA) and Least Square Means. The significance of Least Square Means difference was tested using Dunnett's T-test. Treatment effects were assessed by two-way ANOVA (treatment-sex). When a significant ($p<0.05$) treatment-sex interaction was found, the effect for each sex was evaluated by one-way ANOVA. For continuous data parameters with a pretest value, Analysis of Covariance (ANOCOVA) was used, and the pre-test value was considered the covariate. The statistical methods used for analysis of discrete data (WBC differential) were the same as for continuous data if ANOVA assumptions were satisfied either by direct inspection or via transformation (e.g., arcsine, log). The Mann-Whitney U test and/or log-linear models were used if ANOVA assumptions were not met, and the WBC differential data were treated as discrete.

5. General Observations

(a) Mortality/moribundity/survival

Each animal was observed daily for mortality, signs of ill health, and reaction to treatment. A physical examination was performed weekly on each animal at the time it was weighed. The physical examination included evaluation of the external structure and appearance, posture, gait, behavior, and body orifices.

No treatment-related mortality or clinical signs were observed during the study. One male rat in Group 1 (untreated control) was found dead during week 6 of the study. Necropsy revealed small salivary glands and moderately enlarged mandibular lymph nodes; all organs were soft and darkened as a result of post-mortem autolysis.

(b) Clinical observations

Each animal was observed daily for clinical signs of toxicity. Alopecia, red-stained eyes, and red-stained muzzles were observed sporadically during the study in all groups except Group 1. These observations were judged by the study authors to be caused by the stress of handling and dosing.

(c) Skin irritation

Table 2 describes the Draize Method used in scoring skin irritation.

Table 3 summarizes mean skin irritation scores.

Skin irritation was evaluated daily immediately before each treatment. Erythema and edema were categorized according to the Draize method. In addition, all other skin reactions (e.g., eschar, dessication) were recorded.

A dose-related occurrence of skin irritation was observed in all groups administered RH893 HQ. No occurrence of edema was observed in any of the rats over the treatment period. The first occurrence of skin irritation (erythema) was reported on day 3 in Group 5 males and females. Erythema occurred by day 8 of treatment in all groups administered RH893 HQ. In Group 2 (vehicle control), erythema was not noted until day 52, and then only in males. No erythema was noted for Group 1 (untreated control) on any day.

(d) Body weight/body weight gain/food consumption

Body weight/body weight gain: Table 4 summarizes mean body weight data.

Body weights were determined weekly beginning 1 week prior to dosing. No treatment-related effects on body weight, body weight gain, or cumulative body weight gain were observed in either sex in Groups 2, 3, and 4. No effects on body weight were observed in Group 5 females. In Group 5 male rats, decreased body weight and body weight gain was seen over the 13-week study period. Mean body weights in Group 5 males were statistically significantly decreased as compared to Group 1 males (untreated control) at weeks 2, 3, 6, and 11 of the 13-week treatment period.

TABLE 2. Skin Irritation Scoring Criteria Used in Three-Month Dermal Toxicity Study of RH-893 HQ Technical

A. Erythema and Eschar Formation	
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate- to -severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Total possible erythema score	4
B. Edema Formation	
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (area raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Total possible edema score	4

TABLE 3. Mean Erythema Scores in Rats on Representative Days Following Dermal Application of RH-893 HQ[a,b]

Group No.	Dose Conc.(%)	Dose (mg/kg)	Day of Treatment 1	3	8	21	35	52	65
					Males				
1	0.0[c]	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0[d]	0.00	0.0	0.0	0.0	0.0	0.0	0.1	0.0
3	0.3	2.97	0.0	0.0	0.2	0.1	0.4	0.9	0.9
4	0.6	5.95	0.0	0.0	1.3	0.7	1.2	2.1	1.7
5	1.5	14.87	0.0	0.2	2.6	3.4	3.6	3.1	2.9
					Females				
1	0.0[c]	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0[d]	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.3	2.97	0.0	0.0	0.2	0.0	0.1	0.0	0.4
4	0.6	5.95	0.0	0.0	0.4	0.4	1.4	1.4	0.9
5	1.5	14.87	0.0	0.7	2.3	3.2	3.6	3.7	3.6

[a]Data extracted from Report No. 90R-031, Tables 6A and 6B.
[b]Scoring criteria described in Table 2. Mean edema score equals 0.0 for all groups of either sex on all treatment days.
[c]Untreated control
[d]Vehicle control

TABLE 4. Mean Body Weights (g ± S.D.) at Selected Intervals for Rats Dermally Administered RH-893 HQ for Three Months[a]

Group	Dose Conc.(%)	Observation Period (weeks) 0	2	3	6	11	13	Mean Cumulative Body weight gain 0-13
				Males				
1	0.0[b]	225.3±9.9	321.6±26.2	360.4±29.0	439.9±42.9	523.2±58.3	542.0±68.9	316.6±63.1
2	0.0[c]	226.4±10.2	327.5±16.5	363.2±24.0	446.3±39.9	528.6±61.2	548.6±55.3	322.2±53.1
3	0.3	222.9±6.7	327.4±21.6	369.9±27.5	451.9±38.7	533.7±51.8	563.9±52.6	341.0±53.1
4	0.6	221.5±12.1	304.8±28.6	345.5±36.0	420.2±46.0	490.1±59.2	513.7±66.6	292.2±59.9
5	1.5	222.1±9.4	300.5±15.3[d]	330.1±16.0[d]	401.8±18.4[d]	470.9±23.3[d]	495.7±28.3	273.6±30.5
				Females				
1	0.0[b]	166.2±9.0	213.3±11.7	234.2±14.9	270.5±26.2	302.7±33.7	313.2±38.3	147.0±37.2
2	0.0[c]	159.4±9.9	206.7±12.4	219.2±11.5	248.5±16.0	284.3±19.6	289.3±18.2	129.9±17.6
3	0.3	166.4±10.9	214.0±15.6	234.7±18.5	273.8±23.3	307.9±29.2	312.8±33.8	146.4±25.1
4	0.6	167.7±10.7	208.0±10.3	226.1±10.9	260.6±16.0	290.1±22.4	300.7±24.3	133.0±15.5
5	1.5	159.1±10.6	203.2±20.0	221.7±29.0	256.4±29.6	284.7±37.8	291.9±34.4	132.8±30.2

[a]Data extracted from Report No. 90R-301, Tables 1A and 1B.
[b]Untreated control
[c]Vehicle control
[d]Significantly different from control group 1; $p \leq 0.05$

Terminal body weight was determined for all animals immediately prior to sacrifice. Cumulative body weight was calculated weekly. Body weight gain was determined by subtracting body weight at the start of dosing from weekly body weights.

Mean weekly body weight gain was statistically significantly lower than control Group 1 at week 6 for Group 5 males, at week 8 for Group 4 males, and at week 13 for Group 3 females. Mean cumulative body weight gain was statistically significantly lower than control Group 1 in Group 5 males at weeks 2, 3, 6, and 11.

Food consumption: Food consumption was determined 1 week prior to dosing, and weekly thereafter. Food efficiency was calculated weekly by dividing the weekly change in body weight by food consumption.

Mean food consumption for Groups 2, 3, 4, and 5 was not significantly different from control Group 1. However, food consumption was slightly reduced in Group 5 males, and this reduction may have contributed to the decrease in body weight reported in this group.

The only statistically significant change in food efficiency was a decrease in Group 5 males at week 6 and Group 3 females at week 13.

(e) Ophthalmoscopic examination

An ophthalmic examination was performed on all rats 6 days prior to initiation of treatment and 1 week prior to termination of treatment. There was no indication of test substance-related ocular effects when ophthalmoscopic examination was conducted during the 13th week of treatment.

6. Clinical Pathology

Hematology, clinical chemistry, and urinalysis testing were performed. Blood samples were collected form the abdominal aorta of each rat on the scheduled day of necropsy. Urine samples were freshly voided during week 13 of treatment. The parameters under each study category marked with an X were examined.

(a) Hematology

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
Leukocyte differential count*
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)

* = Recommended by Subdivision F (November 1984) Guidelines

Table 5 summarizes data on selected hematology parameters.

All hematology parameters specified in Guideline 82-3 were examined. There were no treatment-related changes in hematology parameters in any group, with the exception of Group 5 (high-dose) females. In Group 5 (high-dose) females, slight but statistically significant decreases in red blood cell count, hematocrit, and hemoglobin were observed. In males, there was no dose-effect relationship for these parameters. The biological significance of these changes is questionable because although the changes were statistically significant their magnitude was slight and they only occurred in females. Although the authors stated that a differential WBC count was performed, no data were provided.

(b) Blood (clinical) chemistry

Electrolytes

X Calcium*
X Chloride*
X Sodium*
X Potassium*
X Phosphorus*

Other

X Albumin*
X Albumin/globulin ratio
X Blood creatinine*
X Blood urea nitrogen*
X Cholesterol*
X Globulins
X Glucose*
X Total bilirubin*
X Total protein*
X Triglycerides

Enzymes

X Alkaline phosphatase (ALK)
X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*
X Gamma glutamyltransferase (GGT)

* = Recommended by Subdivision F (November 1984) Guidelines

Table 6 summarizes data on selected clinical chemistry parameters.

TABLE 5. Selected Hematology Parameters (Mean ± S.D.) for Rats Dermally Administered RH-893 HQ for Three Months[a]

Group	Dose Conc.(%)	Red Blood Cell Count ($1x10^6/mm^3$)	Hematocrit (%)	Hemoglobin (g/100 mL)
			Males	
1	0.0[b]	8.81 ± 1.10	57.5 ± 6.0	15.5 ± 1.7
2	0.0[c]	9.00 ± 0.45	57.6 ± 2.5	15.4 ± 0.6
3	0.3	9.13 ± 0.51	58.6 ± 2.7	15.8 ± 0.5
4	0.6	8.67 ± 0.93	56.6 ± 6.8	15.3 ± 1.7
5	1.5	9.22 ± 0.43	58.6 ± 2.7	15.8 ± 0.6[d]
			Females	
1	0.0[b]	8.81 ± 0.37	59.0 ± 2.5	15.8 ± 0.5
2	0.0[c]	8.65 ± 0.42	58.1 ± 3.2	15.7 ± 0.8
3	0.3	8.61 ± 0.24	57.9 ± 2.0	15.6 ± 0.4
4	0.6	8.49 ± 0.30	56.7 ± 1.7	15.2 ± 0.6
5	1.5	8.33 ± 0.48[d]	55.4 ± 3.8[d]	15.0 ± 0.7[d]

[a]Data extracted from Report No. 90R-031, Tables 7A and 7B.
[b]Untreated control
[c]Vehicle control
[d]Significantly different from control group 1; $p \leq 0.05$.

TABLE 6. Selected Chemistry Parameters (Mean ± S.D.) for Rats Dermally Administered RH-893 HQ for Three Months[a]

Group	Dose Conc.(%)	Parameter					
		Glucose (mg/dL)	Albumin (U/L)	Total Protein (g/dL)	Alkaline Phosphatase (U/L)	Triglycerides (mg/dL)	Globulin (g/dL)
				Males			
1	0.0[b]	133 ± 19	3.8 ± 0.5	6.0 ± 0.4	167 ± 39	37 ± 17	2.2 ± 0.3
2	0.0[c]	138 ± 14	3.9 ± 0.3	6.0 ± 0.3	156 ± 35	45 ± 19	2.1 ± 0.3
3	0.3	143 ± 18	3.9 ± 0.3	5.9 ± 0.3	172 ± 41	43 ± 15	1.9 ± 0.1[b]
4	0.6	127 ± 40	3.9 ± 0.3	5.9 ± 0.2	197 ± 52	35 ± 20	2.0 ± 0.2
5	1.5	133 ± 14	3.9 ± 0.2	6.0 ± 0.2	208 ± 60[b]	24 ± 07[b]	2.1 ± 0.2
				Females			
1	0.0[b]	149 ± 17	4.5 ± 0.3	6.3 ± 0.3	140 ± 48	43 ± 25	1.8 ± 0.2
2	0.0[c]	142 ± 15	4.3 ± 0.3	6.2 ± 0.3	136 ± 35	24 ± 08	1.9 ± 0.1
3	0.3	141 ± 12	4.3 ± 0.2	6.2 ± 0.2	128 ± 45	29 ± 13	1.9 ± 0.2
4	0.6	132 ± 15	4.4 ± 0.4	6.3 ± 0.3	130 ± 58	29 ± 12	1.9 ± 0.2
5	1.5	126 ± 22[b]	4.0 ± 0.3[b]	6.0 ± 0.3[b]	130 ± 49	23 ± 09	2.0 ± 0.2

[a]Data extracted from Report No. 90R-301, Tables 8A and 8B.
[b]Untreated control
[c]Vehicle control
[d]Significantly different from control group 1; $p \leq 0.05$.

All chemistry parameters specified in Guideline 82-3 were tested. There were no treatment-related changes in chemistry parameters for any group, with the exception of Group 5 (high-dose) males and females. In Group 5 (high-dose) females, statistically significant decreases in albumin, glucose, and total protein were observed. In Group 5 (high-dose) males, an increase in alkaline phosphatase and a decrease in triglycerides were found. The biological significance of these changes is questionable because although the changes were statistically significant, their magnitude was slight and they did not occur in both sexes. Group 3 males demonstrated a statistically significant decrease in globulin; however, in the absence of a dose-effect relationship, this finding was considered incidental by the study authors and not treatment related.

(c) Urinalysis

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

A statistically significant dose-related decrease in specific gravity was observed in Group 5 males. There was no histopathologic evidence of kidney changes in Group 5 males, and no other changes in urinary parameters were observed for any group. Therefore, the biological significance of the change in specific gravity in Group 5 males is questionable.

Guideline 82-3 does not require performance of urinalysis. The Guideline states that urinalysis is to be performed only when there is useful data that might be obtained based on expected or observed toxicity.

7. Sacrifice and Pathology

The checked (X) tissues were collected and preserved in 10% neutral buffered formalin for histological examination; the double-checked (XX) organs were also weighed:

Digestive System	Cardiovascular/Hematologic	Neurologic
X Stomach*	X Aorta*	XX Brain
X Colon*	X Heart*	X Peripheral nerve*
X Duodenum*	X Bone marrow	X Spinal cord (three levels)
X Jejunum*	X Lymph nodes*	X Eyes
X Ileum*	XX Spleen*	X Pituitary*
X Cecum*	X Thymus*	
X Colon*		
X Rectum*	**Urogenital**	**Glandular**
X Salivary glands*		
X Esophagus*	XX Kidneys*	XX Adrenals
XX Liver*	XX Testes*	X Mammary gland
X Pancreas*	X Urinary bladder*	X Thyroids*
	X Epididymides	X Parathyroids*
Respiratory	X Prostate	X Harderian glands
	X Seminal vesicle	
X Lung*	X Ovaries	
X Trachea	X Uterus*	
	X Cervix	
Other	X Vagina	

X Bone (sternum and femur)
X Skeletal muscle
X Normal and Treated Skin*
X All gross lesions and masses*

* = Recommended by Subdivision F (November 1984) Guidelines

Every animal was necropsied over a three-day period, and fasted the night before necropsy. On the day of necropsy, each rat was weighed and anesthetized with an intraperitoneal injection of sodium pentobarbital; two blood samples were then collected from the abdominal aorta. After blood collection, the rats were exsanguinated from the abdominal aorta. Each animal was then necropsied, and all major organs and tissues were examined for gross lesions.

(a) Macroscopic Pathology

Skin: Treatment-related gross changes were observed in the treated area of the skin of male and female rats of all treated groups. Foci were seen in 1 or 2 females in each treatment group and in Group 2 (vehicle control). Foci were seen in 1 male for Groups 2 and 5. Dessication was seen in 8-9 rats of either sex in Groups 4 and 5, and also in 1-3 rats of either sex in Group 3. The study authors suggest that desiccation and multiple raised foci may be attributed to the combined effects of RH893 HQ and the vehicle, propylene glycol. Erythema was seen in 1 rat of each sex

in Group 4 and 2-5 rats of each sex in Group 5. Sores/eschar were seen in 1-2 rats of either sex in Group 4, in 7 females in Group 5, and in 1 male in Group 5. There were no gross changes observed in the area designated as the treated skin of any of the untreated control rats.

Other organs: According to the study authors, the gross changes observed in other organs and tissues were typical of those observed in rats of this age and strain, occurred at comparable incidences among the groups, or occurred sporadically and were not considered to be treatment related.

(b) Organ weights and organ/body weight ratios

Table 7 summarizes selected organ weight data.

No changes in absolute organ weights or relative organ weights (organ/body weight ratios) for males or females were considered to be treatment-related.

Group 5 males demonstrated a statistically significant increase in relative brain weight and a statistically significant decrease in absolute liver weight. A statistically significant increase in absolute brain weight was noted only in Group 3 males; this change was considered incidental and not treatment related.

Group 5 females demonstrated a statistically significant increase in relative adrenal and relative kidney weights.

The study authors stated that the changes in organs weights for Group 5 males and females were not treatment related, but occurred as a result of the slight decrease in terminal body weights in Group 5.

(c) Microscopic pathology

Table 8 summarizes microscopic changes in the skin.

Microscopic pathology was performed on all tissues and gross lesions from the untreated control, vehicle control, and high-dose group rats. For low- and mid-dose rats, microscopic pathology was performed on the kidney, liver, spleen, skin, and gross lesions.

Skin: Treatment-related microscopic changes in the skin were observed in a dose-related manner in Groups 3, 4, and 5. Microscopic changes in the epidermis consisted of hyperkeratosis, acanthosis, necrosis, and eschar formations; in the dermis,

TABLE 7. Selected Organ Weight Data for Rats Dermally Exposed to RH-893 HQ for Three-Months[a]

Group	Dose Conc.(%)	Kidney Weight		Brain Weight	
		Absolute (g)	Relative[b]	Absolute (g)	Relative[b]
			Males		
1	0.0[c]	3.838 ± 0.514	76.344 ± 4.000	2.172 ± 0.121	43.814 ± 6.038
2	0.0[d]	3.868 ± 0.399	75.856 ± 6.995	2.183 ± 0.090	42.950 ± 3.380
3	0.3	4.015 ± 0.465	76.007 ± 3.450	2.275 ± 0.107*	43.367 ± 3.388
4	0.6	3.765 ± 0.581	78.906 ± 13.077	2.212 ± 0.057	46.591 ± 5.377
5	1.5	3.640 ± 0.255	80.485 ± 6.972	2.221 ± 0.081	49.101 ± 2.947*
			Females		
1	0.0[c]	2.277 ± 0.275	78.998 ± 8.166	2.128 ± 0.136	74.372 ± 9.131
2	0.0[d]	2.154 ± 0.185	80.478 ± 6.543	2.081 ± 0.106	77.883 ± 6.071
3	0.3	2.269 ± 0.205	78.557 ± 5.091	2.072 ± 0.137	72.121 ± 7.955
4	0.6	2.313 ± 0.225	84.027 ± 5.707	2.048 ± 0.079	74.672 ± 5.582
5	1.5	2.355 ± 0.212	87.696 ± 5.857*	2.058 ± 0.156	77.092 ± 9.988

[a]Data extracted from Report No. 90R-031, Tables 10A and 10B.

[b]Relative organ weight = organ weight x 10,000/body weight.

[c]Untreated control

[d]Vehicle control

*Significantly different from control group 1; $p \leq 0.05$.

TABLE 7. (Continued)

Group	Dose Conc.(%)	Liver Weight		Adrenals Weight	
		Absolute (g)	Relative[b]	Absolute (g)	Relative[b]
			Males		
1	0.0[c]	13.888 ± 2.529	274.713 ± 21.354	0.072 ± 0.110	1.439 ± 0.253
2	0.0[d]	13.979 ± 1.869	272.874 ± 16.579	0.073 ± 0.010	1.444 ± 0.221
3	0.3	14.404 ± 1.792	272.708 ± 16.124	0.069 ± 0.013	1.310 ± 0.246
4	0.6	13.044 ± 2.013	271.848 ± 27.806	0.070 ± 0.009	1.474 ± 0.243
5	1.5	11.773 ± 1.173[e]	259.410 ± 17.331	0.071 ± 0.013	1.564 ± 0.297
			Females		
1	0.0[c]	8.153 ± 1.164	282.101 ± 28.032	0.072 ± 0.010	2.522 ± 0.424
2	0.0[d]	7.272 ± 0.553	271.386 ± 14.442	0.075 ± 0.012	2.808 ± 0.381
3	0.3	8.077 ± 1.121	277.960 ± 12.872	0.078 ± 0.011	2.730 ± 0.451
4	0.6	7.859 ± 0.676	285.397 ± 12.857	0.076 ± 0.010	2.781 ± 0.392
5	1.5	7.852 ± 0.820	292.073 ± 20.005	0.083 ± 0.013	3.097 ± 0.419[e]

[a]Data extracted from Report No. 90R-031, Tables 10A and 10B.
[b]Relative organ weight = organ weight x 10,000/body weight.
[c]Untreated control
[d]Vehicle control
[e]Significantly different from control group 1; $p \leq 0.05$.

Table 8. Incidence of Skin Histopathology in Rats Dermally Exposed to RH893 HQ for Three-Months[a]

Group No.	Dose Conc.(%)	No. of Rats Examined	Skin Histopathology					
			Hyperkeratosis	Acanthosis	Sebaceous Gland Hyperplasia	Eschar	Necrosis	Chronic Inflammation
				Males				
1	0.0[b]	10	5	5	0	0	0	0
2	0.0[c]	10	9	9	3	1	1	2
3	0.3	10	10	10	9	0	0	2
4	0.6	10	10	10	10	2	2	2
5	1.5	10	10	9	9	4	2	9
				Females				
1	0.0[b]	10	1	1	0	0	0	0
2	0.0[c]	10	1	1	0	0	0	0
3	0.3	10	10	10	3	0	0	0
4	0.6	10	8	8	6	2	1	4
5	1.5	10	10	10	10	10	8	10

[a]Data extracted from Report No. 90R-031, Table 12.
[b]Untreated control
[c]Vehicle control

chronic inflammation and sebaceous gland hyperplasia occurred. The males in vehicle control Group 2 also demonstrated treatment-related changes. When compared to the vehicle control group males, the changes in the treated skin of male rats in the low-dose group (Group 3) were similar except that low-dose males had a higher incidence of sebaceous gland hyperplasia.

A low incidence of hyperkeratosis and acanthosis occurred in untreated control male and female rats, and in vehicle control females. This change was probably due to repeated shaving and washing of the skin.

The study authors stated that in some cases microscopic changes in female rats were of a slightly greater intensity; the method of grading used was not specified. The incidence of microscopic changes in females of the vehicle control group was no different from that of females of the untreated control group.

Other organs: No microscopic changes were observed in any of the other organs or tissues as a result of application of RH-893 HQ technical or the vehicle. The occurrence of microscopic changes in other organs and tissues appeared to be incidental, and not treatment related.

The reviewers have no other comments regarding the materials and methods sections.

A signed Good Laboratory Practice Compliance Statement and a signed Quality Assurance Statement were included.

B. DISCUSSION

The data from this study were well reported, and the summary table data were supported by the data on the individual animals. The study deviated from Guideline 82-3 by not applying an occlusive dressing after dose application; the authors stated that the test site was non-occluded. However, not applying an occlusive dressing is a minor deviation from the Guidance which did not appear to affect the outcome of the study. The authors stated that all rats were fitted with cardboard collars to minimize preening of the application site. Also, RH893 HQ appeared to be nonvolatile and have stability in solution with the vehicle propylene glycol.

Each group consisted of 10 female and 10 male rats as stated in Guideline 82-3 requirements (Group 1 = untreated control group, Group 2 = vehicle control group, Group 3 = dose of 2.97 mg a.i./kg/day RH-893 HQ; Group 4 =

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dose of 5.95 mg a.i./kg/day RH-893 HQ; Group 5 = dose of 14.87 mg a.i./kg/day RH-893 HQ).

The NOEL for systemic toxicity was 5.95 mg a.i./kg/day. A NOEL for dermal irritation was not established. Skin irritation occurred in all treatment groups in a dose-related manner. No mortality or treatment-related signs of clinical toxicity were observed during the study.

Treatment-related microscopic changes were observed in the treated area of the skin of male and female rats in Groups 3, 4, and 5 and in male rats in Group 2 (vehicle control). The incidence and severity of these changes generally occurred in a dose-related manner in the treatment groups. Microscopic changes in the epidermis included hyperkeratosis, acanthosis, foci of necrosis, and eschar formations; chronic inflammation and sebaceous gland hyperplasia occurred in the dermis. Microscopic changes in other organs and tissues were not related to treatment but were considered to have occurred spontaneously.

A treatment-related decrease in body weights and body weight gain in Group 5 (14.87 mg a.i./kg/day) males as compared to Group 1 (untreated control) males was seen at weeks 2, 3, 6, and 11 of the 13-week treatment period. No effects on body weight were observed in Group 5 females. No treatment related effects on body weight, body weight gain, or cumulative body weight gain were observed in either sex in Groups 2, 3, and 4.

No statistically significant effects on food consumption or food efficiency were observed in Groups 2, 3, or 4 of either sex. However, in Group 5 males food consumption was slightly reduced, and this reduction may have contributed to the decrease in body weight reported in this group.

No changes in absolute organ weights or organ/body weight ratios for males or females were considered to be treatment related.

There were no treatment-related changes in hematology parameters in any group, with the exception of Group 5 (high-dose) females. In Group 5 (high-dose) females, slight but statistically significant decreases in red blood cell count, hematocrit and hemoglobin were observed. In males, there was no dose-effect relationship for these parameters. The biological significance of these changes is questionable because although the changes were statistically significant, their magnitude was slight and occurred only in females.

There were no treatment-related changes in clinical chemistry parameters in any group, with the exception of Group 5 (high-dose) males and females. In Group 5 (high-dose) females, statistically significant decreases in albumin, glucose, and total protein were observed. In Group 5 (high-dose)

males, an increase in alkaline phosphatase and a decrease in triglycerides were found. The biological significance of these changes is questionable, because although the changes were statistically significant, their magnitude was slight and they did not occur for both sexes.