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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Systhane or Myclobutanil (RH-53,866 or RH-3866)
EPA Identification Nos. 4G-3149/707-ROG/707-EUP-RNL
CASWELL No. 723K

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Applicant: Rohm & Haas Company
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Submission Purpose

Review the following: Two-generation reproduction study in rats; interim report on testicular pathology in two-year chronic rat and mouse oncogenicity studies, and preliminary findings in one-year dog study; Accession Nos. 073522, 073805, 073806, and 073807; also, review adequacy of labeling changes.

Recommendation

The highest dose (1000 ppm or about 80 mg/kg bwt) of the 2-generation reproduction study was associated with testicular atrophy in the second (P_2) generation, supporting a NOEL of 200 ppm (16 mg/kg bwt). Seminiferous tubular atrophy of the testes was also observed at an increased incidence in the

800 ppm dose level at 12 and 17 months in the interim sacrifices of the 2-year chronic rat feeding study, supporting a similar NOEL to the reproduction study of 200 ppm. No treatment-related increases in testicular atrophy were identified in the 1-year dog study at dietary levels up to 1600 ppm (40 mg/kg bwt) or at 12 months in the mouse oncogenicity study at dietary levels up to 500 ppm (75 mg/kg bwt).

The overall NOEL for the 2-generation reproduction study is 50 ppm (or 4 mg/kg bwt) based on increased absolute and relative liver weights and increased centrilobular hepatic hypertrophy in male rats at 200 ppm (or 16 mg/kg bwt). The 2-generation reproduction study in rats is classified as Core Guideline.

In view of these testicular lesions observed in rats in the reproduction and chronic studies, the registrant has submitted an amendment of the label requiring new protective clothing (long trousers, long-sleeved shirts), impervious gloves, and splash goggles during all methods of mixing, loading, applying, or handling. This new labeling at the present time appears appropriate for purposes of the Experimental Use Permit (EUP).

I. Two-Generation Reproduction Study (Report No. 84R-117; August 21, 1985)

Chemical: RH-3866 or RH-53,866 Technical 84.5% a.i.: α-butyl-α-4-chlorophenyl-1-H-1,2,4-triazole-1-propanenitrile.

Materials and Methods (See Appendix I for details).

Two matings per generation were performed with 25 rats, Sprague-Dawley, (Crl:CD(SD)BR) per sex per group exposed to RH-53,866 at 0, 50, 200, and 1000 ppm. P_1 rats were exposed to compound for 8 weeks prior to mating and through the reproductive phase. The P_2 rats were exposed continuously to compound from conception to at least 8 weeks after weaning and through the reproductive phase.

Clinical signs in the P_1 and P_2 rats were monitored daily. Food consumption and body weights were monitored weekly until cohabitation. Presumed pregnant females were weighed on day 0, 6, 15, and 20 of gestation and lactating dams were weighed on day 0, 4, 7, 14, and 21 of lactation. Necropsies were performed on all P_1 and P_2 animals found dead or killed *in extremis*. Following weaning of the F_{1b} and F_{2b} litters, all surviving P_1 and P_2 rats were subjected to full necropsies and liver to body weight determinations were recorded.

Histopathologic examinations of P_1 and P_2 animals were performed on all gross lesions, on livers, and male reproductive organs in all dose groups. Female reproductive organs were histopathologically examined in the controls and high dose group.

Clinical signs in pups were monitored daily. Body weights, progeny counts, and sex determinations were recorded on lactation day 0, 4, 7, 14, and 21. Pups were randomly culled to 10 pups (5 of each sex) on day 4 and weaned on day 22 of lactation. Necropsies were performed on all F_1 and F_2 pups found dead after 14 days of age.

Results

Diet Analysis and Intake - The average concentration of active ingredient in the samples ranged from 102 percent to 112 percent of the theoretical concentration with an overall mean of 106 percent. The average overall exposure to RH-53,866 in treated groups prior to mating for each generation was calculated on a daily basis to be 4 mg/kg bwt, 16 mg/kg bwt, and 80 mg/kg bwt, for dietary levels 50 ppm, 200 ppm, and 1000 ppm, respectively.

Parental Toxicity

A few deaths occurred at random during the study across all dose groups and were considered unrelated to treatment. Body weight gain for dams was not significantly altered with treatment during the premating, gestation, and lactation periods. Males of the P_2 generation showed 9 percent lower weight gain throughout the 8 weeks prior to mating. A slight reduction in food consumption occurred at the highest dose prior to mating and may reflect a modest degree of poor palatability for the treated chow in both the P_1 and P_2 animals.

Gross Pathology and Liver Weights

P_1 and P_2 animals showed increased absolute liver weights and liver to body weight ratios at the highest dose level in both sexes and at 200 ppm in male rats (see table below).

Liver Weights

	P_1 Abs. Liver Wt (g)	Rel($\times 10^4$) Liver/Body Wt	P_2 Abs. Liver Wt (g)	Rel($\times 10^4$) Liver/Body Wt
Male				
Control	12.77	247	18.2	314
200 ppm	14.56*	264*	19.5*	328*
1000 ppm	14.51*	281*	19.5*	356*
Female				
Control	8.59	289	12.5	367
200 ppm	9.04	295	12.9	373
1000 ppm	9.35*	314*	13.2*	397*

*$p < 0.05$

Gross necropsy also showed an increase in small or flaccid testes at the HDT (8/25) in comparison with controls (0/25) in the P_2 but not P_1 rats.

Histopathology

P_1 and P_2 rats of both sexes at the HDT and males at the 200 ppm level showed evidence of centrilobular hepatic hypertrophy.

Incidence of Centrilobular Hepatic Hypertrophy

	P_1	P_2	
	1000 ppm	1000 ppm	200 ppm
Females	8/25	4/25	
Males	10/25	18/25	2/25

No evidence of hepatic hypertrophy was demonstrated in other treatment groups or controls of the P_1 and P_2 rats (0/25 per group).

Consistent with the gross pathology of small and flaccid testes in the HDT of the P_2 rats, histopathologic examination demonstrated multifocal or diffuse atrophy of the testes in this group at an increased incidence as compared with controls (see below). The high dose males of the P_2 rats also showed increased incidences of epididymal lesions and atrophy of the prostate.

Incidences of Reproductive Lesions in Males

	Dietary Levels				
	Con	Low	Mid	High	
Multifocal or Diffuse Testicular Atrophy	3/25	5/25	5/25	11/25	P_2
	3/25	3/25	3/25	3/25	P_1
Epididymal Lesions					
Necrotic spermatocytes/ spermatids or Decreased spermatozoa	2/25	3/25	3/25	13/25	P_2
	1/25	2/25	0/25	3/25	P_1
Prostate					
Atrophy	2/25	1/25	0/25	11/25	P_2
	2/25	0/25	1/25	2/25	P_1

Reproductive Toxicity

With respect to reproductive parameters, the P_2 generation showed some equivocal increases in reproductive toxicity at the highest dose level evidenced by a slight decrease in the percentage of dams littering (86% vs. 96% in controls) in the P_{2b} mating and a decreased number of pups born per litter (11.4 vs. 13.8 in controls) in the P_{2a}. Reproductive toxicity was more clearly evidenced in the four matings at the high dose (1000 ppm) by an increased number of stillborn or percent born dead.

Number Born Dead (Percent Born Dead)

	Control	1000 ppm
P_{1a}	3 (0.9%)	12* (4.9%)
P_{1b}	0 (0.0%)	16* (4.9%)
P_{2a}	6 (1.9%)	13* (5.7%)
P_{2b}	5 (1.4%)	12 (5.3%)

*$p < 0.05$

Pup Toxicity

Although pup weight was not significantly different between groups at birth, by day 4 in the F_1 pups and by day 7 in the F_2 pups, significant decreases in pup weights were observed in the 1000 ppm group in comparison with controls. The depression of weight gain in the 1000 ppm group in comparison with controls increased as pups matured through day 21 of lactation (see tables 6A-E, in Appendix II).

Discussion and Conclusions

At the highest dose tested, 1000 ppm, reproductive toxicity was most evidenced by an increase in the number of stillborns in each of the two matings per generation. More general toxicity to the parental generations was an increase in absolute and relative liver weights at 200 and 1000 ppm in males and 1000 ppm in females in both P_1 and P_2 rats. These increases in liver weights correlate with the finding of centrilobular hepatic hypertrophy at 1000 ppm in both sexes of P_1 and P_2 rats and in males at 200 ppm in the P_2 rats, suggestive of possible microsomal enzyme induction. P_2 males in the high dose group, which were also exposed to RH-53,866 in utero and during lactation, also showed testicular atrophy, which may as a secondary response to depressed testosterone levels, result in increased atrophy of the prostate and epididymal lesions. P_2 males also demonstrated a decreased body weight gain (9%) prior to mating, suggestive of a greater toxic response to RH-53,866 than the P_1 rats. Finally, weight gain of pups progressively decreased from day 4 through day 21 of lactation in the high dose group in comparison with controls.

The study supports a reproductive NOEL of 200 ppm (16 mg/kg bwt/day) and a LEL of 1000 ppm (80 mg/kg bwt/day), based on: testicular, epididymal, and prostatic atrophy in P_2 males, and in both generations, increased numbers of stillborns, and decreased weight gain in pups during lactation. General systemic toxicity evidenced by increased liver weights and centrilobular hepatic hypertrophy in males at 200 ppm (16 mg/kg bwt/day) support a systemic NOEL of 50 ppm (4 mg/kg bwt/day).

Core Classification: Guideline

II. Interim Report on Testicular Lesions in the Two-Year Chronic Rat Study

In view of the testicular lesions observed in the high dose (1000 ppm) of the P_2 rats in the two-generation reproduction study, an interim report was submitted of testicular weights and histology at the interim sacrifices of 12 and 17 months of the 2-year chronic rat feeding study.

The 10 Sprague-Dawley rats per dose group sacrificed at 3 and 6 months showed no gross lesions of the testes in any treatment or control groups. However, at 12 and 17 months, male rats in the high dose group showed decreased absolute and relative testicular weights. In addition, increased incidences of small testes, identified histologically as unilateral or bilateral testicular atrophy of the seminiferous tubules was observed in the HDT in comparison with controls (see table below).

Table

Testicular Findings in Rat Chronic Study After 12 and 17 Months Exposure to RH-3866

	12 months		17 months	
	Control	800 ppm	Control	800 ppm
Number	20	20	18	18
Testes wt(g)	3.751 ± 0.308	3.300* ± 0.764	3.431 ± 0.914	3.017 ± 1.328
Testes/bwt.	0.556 ± 0.068	0.507 ± 0.157	0.434 ± 0.104	0.389 ± 0.154
Testicular atrophy (unilateral or bilateral)	0/20	4/20	4/18	7/18*

*p <0.05

A more detailed analysis of the testicular lesions at the high dose of the chronic rat study will be performed upon submission of the 2-year terminal sacrifices.

III. Preliminary Results of A One-Year Feeding Study in Dogs (64K-078, Aug 22, MRC)

A preliminary report of a 1-year feeding study in dogs was submitted to assess liver, gall bladder, testicular pathology, and clinical toxicities on RH-3866 for the EUP.

Six males and six female Beagle dogs per treatment and control group were on study for 1 year. Treated groups were exposed to RH-3866 technical in the diet at 10, 100, 400, and 1600 ppm.

Body weight gain, food consumption, and hematology showed no treatment-related significant changes. With respect to clinical chemistries, males in the high dose group showed increased alkaline phosphatase activity and females demonstrate a dose-related increased activity at both 400 and 1600 ppm dietary levels. Slight decreases in serum albumin and small increases of serum phosphorous, especially in males, were also observed at the highest dose level.

Females showed a dose-related significant increase in absolute liver weights and liver to body weight ratios at the 400 and 1600 ppm dietary levels, whereas absolute and relative liver weights were increased in males only at the HDT. Kidney to body weight ratios were significantly increased in males at the two highest treatment levels. Absolute and relative testicular weights were relatively unchanged in treated as compared with control groups.

Preliminary findings of histological examinations were reported for the liver, gall bladder, and testes. Histopathologic examinations of the livers showed centrilobular hypertrophy at the 400 and 1600 ppm treatment levels in both sexes with more severe hypertrophy identified in females (see table below). No histopathological changes were evident in the gall bladder in any group. Finally, no treatment-related increase in incidence or severity of testicular atrophy was evident in dogs exposed for 1 year to RH-3866 at levels up to 1600 ppm in comparison with control males.(see table below).

Histopathology of Liver and Testes in RH-3866-Treated Dogs

	Treatment Level (ppm)									
	Males					Females				
	0	10	100	400	1600	0	10	100	400	1600
Centrilobular hepatic hypertrophy	0/6	0/6	0/6	1/6	5/6	0/6	0/6	0/6	2/6	6/6
Tubular atrophy of the testes	4/6	2/6	1/6	3/6	3/6					

The preliminary findings of the 1-year dog study are consistent with those obtained in the subchronic (3-month) dog study in which the presence of centrilobular hepatic hypertrophy was evidenced at 200 ppm and above in males and 800 ppm and above in females. The 1-year dog study supports a NOEL of 100 ppm based on liver hypertrophy in both sexes and liver weight increases in females at 400 ppm. Moreover, the absence of an increased incidence or severity of testicular atrophy in treated as compared to control dogs at dietary levels up to 1600 ppm (about 40 mg/kg bwt) differentiates the dog from the rat in which testicular atrophy was observed after exposure for 12 months to 800 ppm (about 40 mg/kg bwt) RH-3866. A more complete evaluation of the 1-year dog study will be performed upon receipt of the complete histopathology.

Finally, at the 12-month interim sacrifices of a mouse oncogenicity study, 20 mice in the HDT (500 ppm or 75 mg/kg bwt/day) showed no increased incidence of testicular histopathology or decreased testes weight in comparison to 20 control males.

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