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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

AUG 19 1992

MEMORANDUM

SUBJECT: Kathon® 886 Industrial Microbicide: Submission of a Developmental Toxicity Study to Fulfill the Data Requirements of the Antimicrobial Data Call-In

TO: John Lee/Valdis Goncarovs
Product Manager/PM Team Reviewer (31)
Registration Division (H7505C)
and
Jim Wilson
Product/Review Manager/DCI
Antimicrobial Program Branch
Registration Division (H7505C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 8/13/92
Toxicology Branch II, Section II,
Health Effects Division (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 8/13/92
Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

for Marcia van Gemert, Ph.D. James N. Rowe 8/13/92
Chief, Toxicology Branch II/HFAS/HED (H7509C)

Registrant:	Rohm & Haas Company
Chemical:	5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one
Synonym:	Kathon® 886 Industrial Microbicide
EPA Reg. No.:	707-130
Caswell No.:	195C
Case:	022081
Submission:	S418267
DP Barcode:	D178564
Shaughnessy No.:	107102
Identifying No.:	000707-00130
MRID No.:	423117-01
Action Requested:	Please review study.

Comment: The Registrant has submitted a rabbit developmental toxicity study to fulfill the data requirements for the subject antimicrobial under the Antimicrobial Data Call-In. The study has

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been reviewed and the DER is attached.

Kathon Biocide: Oral (Gavage) Developmental Toxicity Study in Rabbits (MRID # 423117-01). Under the conditions of the study, dose levels of 0.5, 2, 8, and 20 mg Kathon® 886 MW Biocide/kg body weight per day to presumed-pregnant rabbits during Days 7 through 19 of gestation resulted in (1) death and gross pathological lesions of the stomach at the highest dose (100% either died or were sacrificed moribund by Day 15 of gestation), (2) scant or no feces and diarrhea at the 8 and 20 mg/kg dose levels, and (3) decreased body-weight gain, corrected body weight, and food consumption at the 8 mg/kg dose level. The number of implantations, live fetuses, resorptions, dead implants, dead fetuses per doe was comparable among the groups, and fetal body weight (combined and per sex) was comparable among the groups. Although there were no statistically significant differences in the incidence of any fetal alteration (external, visceral or skeletal malformations, variations, or retarded development), there was a tendency for these to occur to a greater degree at the 8 mg/kg dose level than in the control or other treatment groups. The maternal NOEL can be set at 2 mg/kg, the LEL at 8 mg/kg, based on decreased body-weight gain, corrected body weight, food consumption, and scant/no feces and diarrhea. The developmental NOEL can be set at 2 mg/kg, the LEL at 8 mg/kg, based on the slight increase in fetal alterations.

This study is classified Core Minimum and it satisfies the guideline requirement (83-3) for a developmental toxicity study in the rabbit.

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Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor C 8/13/92
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 8/13/92
Section II Head, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity rabbit TOX. CHEM. NO.: 195C

MRID NO.: 423117-01 SHAUGHNESSY NO.: 107102

TEST MATERIAL: Kathon Biocide

SYNONYMS: Kathon 886 Industrial Microbiocide; 75% 5-chloro-2-methyl-4-isothiazolin-3-one and 25% 2-methyl-4-isothiazolin-3-one

STUDY NUMBER: Protocol # 91P-074; Report # 91R-074

SPONSOR: Rohm and Haas Company

TESTING FACILITY: Rohm and Haas Toxicology Department

TITLE OF REPORT: Kathon Biocide: Oral (Gavage) Developmental Toxicity Study in Rabbits

AUTHORS: TL Thomas, HM Solomom, and GP O'Hara

REPORT ISSUED: April 28, 1992

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: Under the conditions of the study, dose levels of 0.5, 2, 8, and 20 mg Kathon® 886 MW Biocide/kg body weight per day to presumed-pregnant rabbits during Days 7 through 19 of gestation resulted in (1) death and gross pathological lesions of the stomach at the highest dose (100% either died or were sacrificed moribund by Day 15 of gestation), (2) scant or no feces and diarrhea at the 8 and 20 mg/kg dose levels, and (3) decreased body-weight gain, corrected body weight, and food consumption at the 8 mg/kg dose level. The number of implantations, live fetuses, resorptions, dead implants, dead fetuses per doe was comparable among the groups, and fetal body weight (combined and per sex) was comparable among the groups. Although there were no statistically significant differences in the incidence of any fetal alteration (external, visceral or skeletal malformations, variations, or retarded development), there was a tendency for these to occur to a greater degree at the 8 mg/kg dose level than in the control or other treatment groups. The maternal NOEL can be set at 2 mg/kg, the LEL at 8 mg/kg, based on decreased body-weight gain, corrected body weight, food consumption, and scant/no feces and diarrhea. The developmental NOEL can be set at 2 mg/kg, the LEL at 8 mg/kg, based on the slight increase in fetal alterations.

Classification: Core Minimum. This study satisfies the guideline requirement (83-3) for a developmental toxicity study in the rabbit.

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A. MATERIALS

1. Test Compound: Kathon® 886MW Biocide; Description: yellow liquid; Batch #: Lot # 32092; Purity: 13.4% a.i. (a.i. is a mixture of 75% 5-chloro-2-methyl-4-isothiazolin-3-one and 25% 2-methyl-4-isothiazolin-3-one; Source: supplied by Jarrow Plant, England, Sample # 89-087.

2. Test Animals: Species: rabbit; Strain: New Zealand White; Age: 5½-6 months old; Weight: females: 3.2-3.4 kg; Source: Hazleton Research Animals, Denver, PA.

3. Statistics: The litter (the proportion of affected fetuses/litter or the litter mean) was considered the experimental unit for the purpose of statistical evaluation. a pair-wise test between the control and treated groups was applied to each parameter. Maternal/corrected body weight, maternal/corrected body-weight gain, and food consumption: Analysis of Variance; when significant results obtained, the Dunnett's test for multiple group comparisons was performed. Incidence of pregnancy, clinical signs, adult necropsy findings, maternal death and litters with total resorptions: Fisher's Exact Test. # Corpora lutea, # implantations, # live fetuses, # dead fetuses, resorptions, mean fetal weight/litter, incidence of fetal alterations: Mann-Whitney U; when more than 75% ties occurred (75% of the litters were unaffected for a particular fetal parameter, then the Fisher's Exact test was used in place of the Mann-Whitney U test to detect significant differences between groups.

B. STUDY DESIGN

Eighty timed-pregnant, nulliparous rabbits (either Day 1 or 2 of gestation on receipt at testing facility) were used in this study and were quarantined during the first two weeks of the study. The rabbits had been naturally mated. Each was housed individually; nesting material was not provided because the does were sacrificed one day prior to expected delivery. Animals were assigned to the groups using a randomization block based on Day 0 of gestation body weight. There were four dose levels (0.5, 2, 8, and 20 mg/kg/day) of the test material (tap water was the vehicle, which control animals received). The test and control materials were administered by oral gavage on Days 7-19 of gestation at a dosing volume of 5 mL/kg of body weight. The total dose administered each day was based on the most recent body weight of each animal. The dose levels were chosen based on the results of a methods development/range-finding study in New Zealand rabbits, a developmental toxicity study in Dutch Belted rabbits (a 1977 IRDC study), and a one-month oral gavage study in Dutch Belted rabbits (1984). None of these studies has been submitted to TB II for review, to date. Throughout the study, the animals were provided with 150 grams of Purina Certified High Fiber Rabbit Chow #5325 and each had free access to filtered tap water.

Dose Preparation: The solutions of test material were prepared daily by stirring an appropriate amount of Kathon Biocide into water; the solutions were continuously stirred prior to and during dosing to ensure homogeneity. Duplicate samples of each dose group plus control

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were taken from early, middle, and late phases of the dosing period for analysis of active ingredient to verify target concentrations.

RESULTS

The data provided show that the concentrations attained were close to the target concentrations (85-103%). No stability data were provided, but it was stated that stability data are available from Rohm and Haas Company study 89R-149.

C. Clinical Observations

Each dam was examined in the afternoon on Days 2-6 of gestation and on Days 20-28 of gestation for morbidity and mortality. Clinical signs were recorded in the morning on Days 2-29 of gestation and also in the afternoon on Days 7-10 of gestation (dosing period). Body weights were recorded on Days 0, 7, 9, 11, 14, 17, 20, and 29 of gestation. Feed was weighed daily between Days 2-29 of gestation.

D. Terminal Procedures

On Day 29 of gestation, all does were sacrificed by an i.v. injection of T-61® via the marginal ear vein. A limited necropsy was performed on each doe, which included an examination for evidence of pregnancy and any gross lesions of the abdominal and thoracic cavities.

E. Uterine/Implantation Data

The uterus of each doe was weighed, opened, and the number and position of live and dead fetuses and resorptions were recorded. Corpora lutea were counted for each ovary. The uterus of each apparently "non-pregnant" rabbit was stained with 10% ammonium sulfide to detect very early resorptions. Data from these rabbits were used only to determine the incidence of pregnancy and the number of litters with total resorptions.

F. Fetal Data

Live fetuses were weighed and examined for external alterations. Each fetus was examined for soft tissue alterations using the Staples technique and the sex of each fetus was determined. The brain was examined by making a transverse section (between the parietal and frontal bones) through the unfixed fetal head. The eyes of each fetus were examined visually to detect microphthalmia. All fetuses were fixed in 95% ethanol, macerated in 2% aqueous potassium hydroxide solution and stained with alizarin red S for skeletal examinations.

RESULTS

1. Clinical Observations and Survival - Maternal

All does at the highest dose level (20 mg/kg) either died or were sacrificed moribund; one death at this dose level was attributed to dosing error. One 8 mg/kg doe also died, and this was attributed to

dosing error also. There were no deaths at either of the two lower dose levels. None of the does aborted or delivered prematurely. Scant feces, no feces, and diarrhea were observed at the 20 mg/kg dose level, and treatment-related increases in scant feces were noted at the 8 mg/kg dose level.

2. Maternal Body Weight and Body-weight Gain

With the exception of the 20 mg/kg dose group, body weights were comparable among the groups during gestation [low- (0.5 mg/kg) and mid-dose (2 mg/kg) groups were 96% of control value; 8 mg/kg dose (considered the high-dose since all 20 mg/kg {HDT} does died) was 90-96% of control]. Body-weight changes varied considerably among the groups at different time intervals. The high-dose (8 mg/kg) does displayed lower gains during most of the intervals measured (see table below). All treated does displayed gains equal to or greater than the control group prior to dosing (days 0-7); the high-dose group displayed a negative gain during days 7-9 and 9-11 of dosing and for the dosing period (days 7-20).

Body-weight Gains [grams]

Dose (mg/kg) Time Interval	0	0.5	2	8
0-7	19.1	19.3	27.5	34.9
7-9	7.3	8.5	-7.4	-59.6*
9-11	-5.7	-2.3	21.9	-63.6*
11-14	130.2	118.6	99.7	19.6*
14-17	33.0	36.7	38.1	71.1
17-20	14.5	13.1	-0.6	41.0
20-29	204.3	171.7	165.5	219.8
0-29♦	375.6	356.1	368.9	264.7
7-20	188.5	179.9	141.5	-2.9*

♦ calculated by TB II, no statistics performed; * $p<0.05$

Gravid uterine weight was comparable among the groups, although a slightly lower (89% of control) weight was observed at the 2 mg/kg dose level compared to the control. The corrected body weights were comparable, although the 8 mg/kg dose level displayed a greater negative change from day 7 of the study (see table below) compared to the control, which is considered biologically significant, although a $p<0.05$ was not attained.

Uterine and Net Body Weights (g)

Dose (mg/kg) Parameter	0	0.5	2	8
gravid uterus	545.2	494.3	482.9	516.9
corrected body weight	3217.8	3109.9	3124.2	2973.7
net weight change from day 7	-181.8	-157.6	-141.5	-293.9

NOTE: Body weight data for the 20 mg/kg dose group were not presented in the text of the report, nor were any calculations performed. It is to be noted that this group gained very little weight during the week prior to the start of dosing, and a higher percentage of the does in this group lost weight during this period compared to the control and other treatment groups. The percent of does losing weight during the week prior to study start was 38%, 43%, 27%, 36% and 56% for the 0, 0.5, 2, 8, and 20 mg/kg dose groups, respectively.

Body-weight change (g) - 20 mg/kg does

Days 0-7	5.9 (31% of control)
Days 7-9	-229.7

3. Food Consumption

The high-dose group (8 mg/kg) displayed a statistically significant decrease in mean daily food consumption compared to the control value throughout the dosing period.

Food Consumption [grams/animal/day]

Dose (mg/kg) Days	0	0.5	2	8	20♦
2-7	141.9	141.8	145.4	148.0	140.4
7-9	150.0	149.8	146.8	101.0*	8.4
9-11	147.7	149.6	150.0	69.9*	-
11-14	147.5	146.8	148.6	79.2*	-
14-17	145.6	146.0	149.6	112.3*	-
17-20	144.6	148.8	150.0	134.7	-
20-29	147.7	141.2	145.9	146.2	-
7-20	146.8	148.0	149.1	101.5*	-

♥ values are those given in report, except for the 20 mg/kg group;
♦ calculated by TB II, no statistics; * p≤0.05

4. Gross Pathological Observations

There were no treatment-related findings observed at the 0.5, 2, and 8 mg/kg dose levels. At the 20 mg/kg dose level, thickened stomach, reddened stomach, and necrotic stomach were observed, indicating local irritation.

5. Maternal Observations

The highest number of non-pregnant animals occurred in the control group. The numbers of corpora lutea, implantations sites, and live fetuses were comparable among the groups. Pre-implantation loss was slightly increased at the 2 and 8 mg/kg dose levels compared to the control and 0.5 mg/kg dose groups. Post-implantation loss was comparable among the groups. The Cesarean section observations are listed below.

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Table I (a): Cesarean Section observations (all does)

GROUP:	CONTROL	0.5	2	8	20
# Females mated	16	16	16	16	16
# Pregnant Females	13	15	16	15	16
Pregnancy Rate (%)	81.3	93.8	100	93.8	100
Maternal Wastage					
#Died	0	0	0	1	16
#Died/pregnant	0	0	0	1	16
#Non pregnant	3	1	0	1	0
#Aborted	0	0	0	0	0
#Premature Delivery	0	0	0	0	0
# Females with 100% intrauterine deaths	0	1	1	0	0
# Females with live fetuses at necropsy (%)	13 (81)	14 (88)	15 (94)	14 (88)	xx
Total # Corpora Lutea	134	137	144	149	xx
Corpora Lutea/dam	10.3	9.8	9.6	10.6	xx
Total # Implantation	123	124	125	129	xx
Implantations/Dam	9.5	8.9	8.3	9.2	xx
Total # Live Fetuses	114	121	118	122	xx
Live Fetuses/Dam	8.8	8.6	7.9	8.7	xx
% of implantations					xx
Total Resorptions	9	3	6	6	xx
Resorptions/Dam	0.7	0.2	0.4	0.4	xx
Early	0.2	0.1	0.1	0.2	xx
Late	0.5	0.1	0.3	0.2	xx
# Litters w/ resorptions (%)	5(38)	3(21)	5(33)	4(29)	xx
mean litter %	7.3	2.8	4.4	4.0	xx
Total # Dead Fetuses	0	0	0	1	xx
Dead Fetuses/Dam	0	0	0	0.07	xx
Postimplantation Loss(%)	7.4	2.2	6.0	5.4	xx
Preimplantation Loss(%)	7.4	4.9	11.9	12.0	xx
Litter Weight (gm)	85.1	80.7	85.1	80.3	xx
Mean Fetal Weight (gm)	42.5	40.1	42.6	40.2	xx
Mean Male Weight	42.8	41.2	42.8	40.4	xx
Mean Female Weight	42.3	39.5	42.3	39.9	xx
Sex Ratio (% Male)	50.3	53.8	48.9	56.4	xx

6. Fetal Observations

The number of fetuses/litter was comparable among the groups. Litter weight was unaffected by treatment. The mean fetal body weights of the male and female fetuses were comparable among the groups and between the sexes, and the sex ratio was comparable among the groups.

Mean Fetal Body Weight (grams)

GROUP/SEX	MALES	FEMALES	LITTER♦
CONTROL	42.8	42.3	365.3
LOW DOSE	41.2	39.5	344.0
MID DOSE	42.8	42.3	328.3
HIGH DOSE	40.4	39.9	346.1

♦ data calculated by TB II

There were no statistically significant increases observed in the type or incidence of external, visceral, or skeletal malformations at any dose level. Those malformations observed were considered random and are commonly seen in rabbit fetuses. Additionally, there were no statistically significant increases in the type or incidence of developmental variations, variations due to retarded development, or in the total of these two categories at any dose level compared to the control (TABLE I).

There were no fetal external malformations. Fetal soft tissue malformations were observed [only at the high-dose level (8 mg/kg)] in three fetuses from three litters (one fetus with microphthalmia and two fetuses with pulmonary artery (vestigial and agenesis of semilunar values), heart (ventricular septal defect), and aorta (ascending and transverse enlarged) malformations. Only one fetus (2 mg/kg dose group) displayed a skeletal malformation (fused sternebra).

There were no fetal external variations, and the soft tissue variations showed no dose response. Skeletal variations showed no statistically significant increase in incidence, although there was a slight increase in the incidence of extra ribs at the 8 mg/kg dose level (58% of the fetuses were affected, 100% of the litters) compared to the control (39% of the fetuses affected, 85% of the litters). The mean litter % (calculated by TB II, no statistics performed) was increased compared to the control value (53.2 vs 35.8).

There was no fetal external or soft tissue retarded development in any group, but skeletal retarded development was displayed in all groups, with the 8 mg/kg dose group displaying a slightly increased incidence ($p<0.05$ was not attained).

TABLE I. FETAL DEFECTS

GROUP:	CONTROL	LOW	MID	HIGH
# Litters	13	14	15	14
# Fetuses (# live fetuses)	114	121	118	122
EXTERNAL/VISCERAL MALFORMATIONS				
Microphthalmia				
# litters	0	0	0	1 (1.7%)
# fetuses	0	0	0	1 (0.8%)
mean litter %	0	0	0	0.71
Pulmonary artery, vestigial				
# litters	0	0	0	2 (14.3%)
# fetuses	0	0	0	2 (1.6%)
mean litter %	0	0	0	1.3
Aorta, ascending & transverse enlarged				
# litters	0	0	0	2 (14.3%)
# fetuses	0	0	0	2 (1.6%)
mean litter %	0	0	0	1.3
Heart, ventricular septal defect				
# litters	0	0	0	2 (14.3%)
# fetuses	0	0	0	2 (1.6%)
mean litter %	0	0	0	1.3
Pulmonary artery, semilunar valves, agenesis				
# litters	0	0	0	2 (14.3%)
# fetuses	0	0	0	2 (1.6%)
mean litter %	0	0	0	1.3
SKELETAL MALFORMATIONS				
Sternebra, fused				
# litters	0	0	1 (0.8%)	0
# fetuses	0	0	1 (6.7%)	0
mean litter %	0	0	0.7	0
SKELETAL VARIATIONS				
Rib, extra				
# litters	11 (85%)	13 (93%)	14 (93%)	14 (100%)
# fetuses	45 (39%)	61 (50%)	47 (40%)	71 (58%)
mean litter %	35.8	50.6	39.4	53.2
SKELETAL RETARDED DEVELOPMENT				
sternebra, partially ossified				
# litters	12 (92%)	10 (71%)	10 (67%)	12 (86%)
# fetuses	30 (26%)	24 (20%)	34 (29%)	44 (36%)
mean litter %	25.1	19.6	28.8	33.9

The table below (TABLE II) summarizes the total fetal alterations observed in each group as presented by the author: (a) number of litters in each group displaying defect; (b) number of fetuses in each group displaying the defect; and (c) mean litter percent displaying the defect.

TABLE II. TOTAL FETAL ALTERATIONS

GROUP:	CONTROL	LOW	MID	HIGH
MALFORMATIONS				
Fetal incidence	0	0	1	3
Litter incidence	0	0	1	3
mean litter %	0	0	0.74	2.16
VARIATIONS				
Developmental				
Fetal incidence	66	80	72	83
Litter incidence	11	14	15	14
mean litter %	57.5	66.4	62.0	67.8
Retardations				
Fetal incidence	49	34	54	60
Litter incidence	12	10	12	14
mean litter %	42.6	28.8	42.4	49.2
TOTAL VARIATIONS				
Fetal incidence	91 (80%)	91 (75%)	95 (81%)	105 (86%)
Litter incidence	13	14	15	14
mean litter %	79.8	76.0	79.9	85.5

D. Discussion

All does at the highest dose administered (20 mg/kg) died or were sacrificed moribund between days 10 and 15 of dosing. Clinical signs (scant or no feces and diarrhea) were observed at the 8 and 20 mg/kg dose levels (dose-related). Maternal body weight gains and food consumption were decreased at the 8 mg/kg dose level compared to the controls during the dosing period, and the corrected body weight was decreased at this dose level also.

The control does had the highest number of non-pregnant animals and consequently, the lowest pregnancy rate (81.3 vs 93.8 & 100%) compared to the treated groups. There was no treatment-related effect on the number of corpora lutea/implantations/live fetuses per doe, and pre- and post-implantation loss were comparable among the groups. There was no dose-related effect on the number of fetuses/doe, mean fetal weight (combined or per sex), fetal sex distribution, or fetal mortalities.

There was no statistically significant increase in the incidence of any external, visceral or skeletal malformations, variations, or retarded development at any dose level. However, of the fetal alterations observed, there was a tendency for these to occur to a greater degree at the high (8 mg/kg) dose level than in the control or other treatment groups.

D. CONCLUSION

Under the conditions of the study, dose levels of 0.5, 2, 8, and 20 mg Kathon® 886 MW Biocide/kg body weight per day to presumed-pregnant rabbits during Days 7 through 19 of gestation resulted in (1) death and gross pathological lesions of the stomach at the highest dose (100% either died or were sacrificed moribund by Day 15 of gestation), (2) scant or no feces

and diarrhea at the 8 and 20 mg/kg dose levels, and (3) decreased body-weight gain, corrected body weight, and food consumption at the 8 mg/kg dose level. The number of implantations, live fetuses, resorptions, dead implants, dead fetuses per doe was comparable among the groups, and fetal body weight (combined and per sex) was comparable among the groups. Although there were no statistically significant differences in the incidence of any fetal alteration (external, visceral or skeletal malformations, variations, or retarded development), there was a tendency for these to occur to a greater degree at the high (8 mg/kg) dose level than in the control or other treatment groups. The maternal NOEL can be set at 2 mg/kg, the LEL at 8 mg/kg, based on decreased body-weight gain, corrected body weight, food consumption, and scant/no feces and diarrhea. The fetal NOEL can be set at 2 mg/kg, the LEL at 8 mg/kg, based on the slight increase in fetal alterations. This study is classified Core minimum, and it satisfies the guideline requirement (83-3) for a developmental toxicity study in the rabbit.

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