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DATA EVALUATION REPORT

STUDY TYPE: Chronic Toxicity/Carcinogenicity-rat PC Code: 107103 & 107104

MRID NO.: 431407-01

TEST MATERIAL: Kathon® Biocide 011287

SYNONYMS: Kathon® 886F Industrial Microbicide

CHEMICAL NAME: 5-chloro-2-methyl-4-isothiazolin-3-one + 2-methyl-4-isothiazolin-3-one

STUDY NUMBER: Report # 90R-149

SPONSOR: Rohm and Haas Company

TESTING FACILITY: Rohm and Haas Company Toxicology Department

TITLE OF REPORT: Kathon® Biocide: 24-Month Drinking Water Chronic/ Oncogenic Study in Rats

AUTHOR(S): DL Quinn, GP O'Hara, and WR Brown

REPORT ISSUED: January 24, 1994

EXECUTIVE SUMMARY: Under the conditions of the study, exposure of Crl:CD®BR rats [90 males/80 females/group to Kathon® Biocide via the drinking water at dose levels of 30 ppm [♂♂ 2.0/♀♀ 3.1 mg/kg], 100 [♂♂ 6.6/♀♀ 9.8 mg/kg], and 300 [♂♂ 17.2/♀♀ 25.7 mg/kg], or to tap water or 0.5% salt solution [$MgCl_2$ and $Mg(NO_3)_2$] vehicles for 24 months resulted in a dose-related decrease in drinking water consumption at all dose levels in both sexes throughout the study. Decreased body weight [95-98% of control]/body weight gain [87% of control at week one, from 91-98% thereafter] were observed in the high-dose males, although statistical significance was not always attained. Females displayed an equivocal decrease in body weight throughout the study, with the mid- [93-96% of control] and high-dose [87-96% of control] groups showing comparable decreases that were not always dose-related. During the second year, body-weight gains of the high-dose females were significantly decreased [83-88% of control]. High-dose males displayed a significant decrease [91-98% of control] in food consumption compared to the control groups throughout most of the study, and females at all dose levels displayed significant decreases in food intake but a dose response was not always evident. The hematology and clinical chemistry parameters monitored were comparable among the groups of both sexes, as were the organ weights and eyes. During the first 6 months of the study, the specific gravity of the urine was increased in both sexes, which may be attributed to the decrease in water consumption. With the exception of the stomach, none of the gross and non-neoplastic lesions observed could be attributed to treatment. An increased incidence in hyperplasia and hyperkeratosis of the squamous mucosa of the stomach was observed at the mid-

and high-dose levels in both sexes, which correlated with the finding of prominence of the limiting ridge and/or thickened nonglandular mucosa of the forestomach on gross examination. Additionally, there was an increased incidence of necrosis of the glandular mucosa in the mid-dose females and in both sexes at the high-dose level. There was no treatment-related increase in the incidence of any tumor in either sex. **The NOEL can be set at 30 ppm [♂♂ 2.0/♀♀ 3.1 mg/kg/day], LEL at 100 ppm [♂♂ 6.6/♀♀ 9.8 mg/kg/day], based on microscopic lesions [hyperplasia/ hyperkeratosis in both sexes, necrosis of glandular mucosa in females] in the stomach.** This study is classified Core Minimum, and it satisfies the guideline requirement (83-5) for a combined chronic toxicity/ carcinogenicity study in a rodent.

A. MATERIALS

1. Test Compound: Kathon® biocide; Description: light brown liquid; Batch #: Lot #: 48014; Purity: 14.2% of the following active ingredients: 5-chloro-2-methyl-4-isothiazolin-3-one [10.6%] and 2-methyl-4-isothiazolin-3-one [3.5%]. Inorganic 'salt' control: aqueous $MgCl_2/Mg(NO_3)_2$ salt solution; Description: clear liquid; Batch #: Lot #: JMG-7-3-1 and Lot # JG-0704; Purity: 9.1% $MgCl_2$/15.4% $Mg(NO_3)_2$ and 9.0% $MgCl_2$/ 16.0% $Mg(NO_3)_2$.

2. Test Animals: Species: Rat; Strain: Crl:CD®BR; Age: ≈3 weeks old on receipt; ≈ 6 weeks old at study initiation; Weight: 201-205 g ♂, 158-164 g ♀ at study initiation; Source: Charles River Laboratories, Portage Facility, Portage MI.

3. Statistics: Prior to the analysis of treatment-related effects, the 'salt' control and tap water control groups were compared using 2-sample Student t-tests to determine any effect of the salt constituents [$MgCl_2$ and $MG(NO_3)_2$] of Kathon ® Biocide on the various parameters monitored. Data were pooled across control groups for body weight, cumulative body-weight change, water consumption, and feed consumption when no significant difference was attained for most time points. Tests for treatment effects for these parameters used pooled control data. Data were pooled also for hematology, clinical chemistry, urinalysis, and organ weight data if no significant difference between the control groups was attained, and treatment effects were compared to the pooled data also. When a significant difference was attained between the control groups, the Kathon® biocide groups were compared to the 'salt' control. Body weight, cumulative body weight change, water and feed consumption, hematology, clinical chemistry, urinalysis, and organ weights: inspected for normality and homogeneity of variance across treatment groups and sampling times. Square root transformations were used for parameters of the white blood cell differential prior to further analysis. analysis of variance [or covariance if pre-test scores were available] were used to determine whether significant differences existed among the various treatment group means. When significant differences were found, pairwise comparisons of least square means were made between each Kathon® biocide-treated group and control using Dunnett's t-test. Survival data: analyzed by life table techniques consisting of Kaplan-Meier product limit estimates, Cox-Tarone binary regression on the life tables, and Gehan-Breslow nonparametric methods. Nonneoplastic findings: analyzed by Cochran-Armitage method for trend and Fisher-Irwin exact for control vs treatment comparisons. Neoplastic lesions: initially analyzed as for the non-neoplastic lesions; when intercurrent mortality differences among treated groups occurred, survival adjusted tumor analyses were performed using logistic regression of tumor prevalence. Ordinal dose levels were used in order to obtain continuity-corrected-one-sided probability levels for trend. Trend probabilities were evaluated when there was any monotone response in these data. Continuity corrected test statistics were used for evaluation of all the incidence tables, where appropriate. In the absence of any valid monotone trend, control vs treated comparisons were made using Bonferroni adjustment to significance levels.

B. STUDY DESIGN

1. Animal Assignment: One thousand and 98 rats were purchased for this study. Four hundred and ninety male and four hundred and forty female healthy rats were assigned to the study. The rats were assigned randomly [computer-generated randomization] to one of two control groups [90 ♂♂/80 ♀♀ per group], one of three Kathon® biocide-treated groups [90 ♂♂/80 ♀♀ per group], or to the sentinel group [40 rats/sex]. The test material was administered via the drinking water. The control groups were provided with either untreated filtered tap water or tap water containing the same concentration of inorganic stabilizer salts [$MgCl_2$ and $Mg(NO_3)_2$] found in the high-dose Kathon® solution [0.05%]. The Kathon® biocide dosing solutions were tap water containing the test material at 30, 100, and 300 ppm. The sentinels received untreated filtered tap water. The selected rats were quarantined/acclimated to the study room for ≈3 weeks following arrival at the testing facility. After the first week of the acclimation period, the rats were housed individually. All rats had access to Purina® Certified Rodent Chow® #5002 meal as well as to the control or treated dosing solutions ad libitum.The rats were administered the dosing solutions for 24 months, and there were two interim sacrifices, one at 12 and one at 18 months [10 rats/sex/group].

Prior to group assignment, 6 rats/sex were selected randomly for comprehensive health monitoring, including serology, bacteriology, parasitology, and pathology. No adverse findings were noted. An additional 100 male rats were selected randomly, euthanized, and necropsied to determine the incidence of renal pelvic dilation in this lot of rats, since a previous chronic study [not submitted to TB II for review] had to be terminated prematurely [at 6 months] due to a high incidence [≈30%] of hydronephrosis in the males in that study. The incidence [3%] in the current lot of males was determined to be acceptable. The sentinels in the current study were monitored for intercurrent disease and the health status of the rats throughout the study. At the 6- and 18-month intervals, 20 randomly-selected sentinels [10/sex] were euthanized, and tissues and blood samples were collected for standard health monitoring. At the 12-month interval, 10 rats/sex were selected for the comprehensive health monitoring described above.

2. Diet Preparation: Dosing solutions [both the salt control and Kathon® biocide] were prepared once a week for each week of the study. Appropriate amounts Kathon® biocide [≈14.2% a.i.] or aqueous $MgCl_2/Mg(NO_3)_2$ solution for each dosing solution were weighed, dissolved in an appropriate amount of filtered tap water, and the solutions were stirred to ensure homogeneity. Fresh dosing solutions were provided once every 7 days or as needed. Week one dosing solutions were analyzed for homogeneity [top, middle, and bottom samples of each dosing solution] and concentration, and tap water was analyzed to ensure no cross-contamination. Single samples from each dose level were analyzed for concentration for weeks 2-4 of the study, and at the end of the first 4 weeks, samples were analyzed to determine stability in tap water. Weekly samples were collected from each dose level, and one set of samples was analyzed monthly for concentration.

RESULTS

The results of the analyses for homogeneity showed that the mixing procedure was adequate. The concentrations attained throughout the study were satisfactory, with greater than 92% of target being attained overall. In the text of the report, it is stated that the test material was determined to be stable in tap water for 9 days at room temperature, but this reviewer was not able to locate any data showing results from samples stored for different lengths of time [Appendix 17]. However, since the analyses of the dosing solutions throughout the 2-year study showed a consistent attainment of the target dose levels, and samples were stored at room temperature until analyzed, it can be assumed that stability was not a problem.

C. METHODS AND RESULTS

1. Observations

Daily observation of each animal was performed for signs of toxicity or ill health. Cage liners were inspected for abnormal appearance of urine or feces and excessive spillage of dosing solutions. Weekly physical examinations [evaluation of external structures, posture, gait, behavior, abnormalities in respiration or body temperature] were performed from one week prior to study initiation throughout the study. All rats were palpated for tissue masses.

Toxicity/Mortality (survival)

Survival rates were comparable among the groups for both sexes. The percent survival for all groups [adjusted for scheduled interim sacrifices] is listed below. NOTE: The numbers of nonsurvivors in the female control, mid-, and high-dose groups reported in the Pathology Report in Appendix 14 [page 3153] differ from those reported on pages 51 and 53 of the study report. There were no apparent differences observed in the clinical signs observed during the study.

Table 1. Survival (%)

Parameter/Treatment(ppm)/ Sex	MALES	FEMALES
percent		
0	36	35
salt♥	41	35
30	30	33
100	37	38
300	46	32
# nonsurvivors [pg. 51/53]		
0	46	39
salt♥	43	40
30	51	40
100	45	37
300	40	40
# nonsurvivors [pg. 3153]		
0	46	40
salt♥	43	40
30	51	40
100	45	39
300	40	41

♥ $MgCl_2/Mg(NO_3)_2$

2. Body Weight: Individual body weights were determined weekly from one week prior to study initiation through the 13th week of the study; thereafter, weights were measured once every 4th week through week 101. Group mean body weights were calculated weekly for the first 13 weeks and then once every 4th week.

RESULTS

MALES - Throughout the study, males at the high-dose level displayed statistically significant decreases in body weight and body-weight gains compared to the control values, although the differences were slight [Tables 2 & 3]. No significant differences in either body weight or body-weight gain were displayed at the mid- and low-dose levels at any time point.

FEMALES - Females at the two highest dose levels displayed statistically significant decreases in body weight and body-weight gains compared to the control values on numerous occasions, although a dose response was not always evident and the differences were slight [Tables 2 & 3]. The low-dose females also displayed statistically significant decreases in body weight [weeks 57 and 65]/gain [week 57]. It is noted that the salt control females displayed significantly increased body weights/gains compared to the tap water controls on several occasions. Since pooled control data were utilized, and the decrease was greater at the mid-dose level during the first year, the effect appears equivocal.

Table 2. Body Weight (% of combined control)

Week/Dose	salt	30 ppm	100 ppm	300 ppm
MALES				
Pre-test	100	102	102	101
1	99	102	101	99*
2	100	102	101	98*
3	100	102	101	98*
4	100	102	101	99*
5	99	102	101	99
6	99	102	101	99
13	100	103	101	98
17	100	102	100	98*
33	99	102	100	97*
53	100	103	99	96*
81	101	104	99	95*
89	102	103	100	95
101	107	102	100	100
FEMALES				
Pre-test	100	98	97	97
1	101	99	96	96*
2	101	99	96	96*
3	101	99	97	96*
4	102	99	97	97
10	102	99	96*	97
11	102	99	96*	97
13	103	99	96*	97
17	103	98	95*	97
41	106	98	95*	96*
53	106	98	95*	94*
57	104	94*	95*	93*
61	104	95	95*	91*
65	103	94*	93*	91*
81	97	95	92	88*
93	97	92	91	87*
101	103	102	95	91

* $p<0.05$

Table 3. Mean Cumulative Body-Weight Change [g (% of combined control)]

Week/Group	0 ppm	salt	30 ppm	100 ppm	300 ppm
MALES					
1	50	49	51	49	44*(87)
2	97	98	99	98	88*(91)
3	132	132	135	134	124*(94)
4	161	161	165	163	154*(95)
5	188	187	193	190	181(96)
6	213	210	218	213	205(96)
13	309	312	319	310	298(96)
25	389	389	402	391	378(97)
45	468	465	485	462	442*(94)
53	477	478	491	468(98)♦	445*(93)
57	484	487	491	467(96)	452*(93)
77	509	518	529	498(98)	477*(94)
93	488	519	502	490	471(96)
101	483	522	485	472(98)	472(98)
FEMALES					
1	21	22	22	19(92)	18*(86)
2	39	41	40	37(95)	35*(90)
3	55	58	55	53(96)	52*(94)
4	67	71	67	65(97)	64(96)
9	110	113	108	103*(94)	108(99)
10	117	122	116	110*(94)	114(98)
13	125	133*	124	119*(96)	121(97)
25	162	174*	162	152*(94)	161
45	211	231*	206	198*(94)	198*(94)
53	225	247*	220	211*(94)	210*(93)
57	243	259	223*(92)	219*(90)	220*(90)
61	247	263	229(93)	224*(91)	218*(88)
69	266	281	243(91)	244(92)	231*(87)
77	275	288	257(93)	257(93)	238*(86)
93	337	326	301(89)	298(89)	279*(83)
101	331	348	344	310(94)	297(90)

* $p<0.05$; ♦(% of control value)

3. Water, Food Consumption, and Compound Intake

The quantity of water/dosing solution and feed consumption were determined weekly from one week prior to study start through the 13th week of the study; thereafter, these parameters were measured once every 4th week through week 101. Food and water consumption were calculated weekly for the first 13 weeks and then once every 4th week. Test material intake was calculated as follows:

$$\text{Kathon® intake (mg/kg/day)} = \frac{\text{Concentration in } H_2O \text{ (ppm) x Water Consumption (g/rat/day)}}{\text{Body Weight (grams) at End of Week}}$$

RESULTS

The overall mean daily intake values of test material for both sexes are listed below (Table 4).

Dose (ppm)	Table 4. Mean Daily Kathon® Biocide Intake [mg/kg/day]	
	MALES	FEMALES
30	2.0	3.1
100	6.6	9.8
300	17.2	25.7

Water consumption was decreased [dose-related] in both sexes at all dose levels of Kathon® Biocide throughout the study compared to the

tap water control, and the decrease was attributed to the unpalatability of the test material [Table 5]. At a few time points, the salt control displayed values that were statistically significantly increased or decreased from the tap water control groups.

Table 5. Water Consumption [% of tap H_2O control]

Week/Group/Dose	salt	30 ppm	100 ppm	300 ppm
MALES				
0	99	101	102	102
1	101	93*	88*	75*
2	102	93*	86*	70*
3	107*	94*	90*	75*
4	106*	96*	91*	73*
5	106	94*	89*	74*
6	99	90*	86*	72*
7	99	93*	85*	72*
8	96	91*	86*	72*
9	102	91*	84*	75*
10	102	91*	83*	71*
11	101	91*	84*	73*
12	105	95*	88*	74*
13	102	91*	88*	74*
17	98	94*	91*	73*
25	104	92*	89*	78*
53	100	94*	92*	79*
89	97	93	88*	82*
101	96	99	88	84*
FEMALES				
0	100	97	94	100
1	101	87*	74*	69*
2	106	91*	81*	69*
3	108*	92*	80*	73*
4	108	92*	78*	67*
5	102	81*	74*	66*
6	96	81*	70*	63*
7	100	83*	74*	64*
8	103	91*	77*	65*
9	100	88*	79*	64*
10	101	85*	73*	63*
11	99	81*	73*	60*
12	93	84*	77*	64*
13	103	88*	78*	66*
17	98	90*	77*	67*
25	101	85*	78*	69*
53	99	90*	82*	75*
81	72*	82	76	70*
89	80*	78*	82	71*
93	84*	84	76*	71*
101	85	102	87	78

* $p<0.05$

A significant decrease [from 91-98% of control] in food consumption was observed in males at the high-dose level throughout most of the study [Table 6]. Females displayed decreased [from 83-97% of control] food intake at all dose levels at several time points throughout the study, but a dose response was not always evident [Table 6].

Table 5. Mean Food Consumption [g/rat/week; (% of tap H_2O control)]

Week/Group	salt	30 ppm	100 ppm	300 ppm
MALES				
0	98	101	100	100
1	99	101	98	91*
2	100	102	100	95*
3	100	102	101	95*
4	100	101	100	97*
5	100	101	101	98*
6	98	101	99	97
9	97*	99	98	98
13	99	102	101	98
53	100*	101	101	94*
73	107*	100	105	97*
89	99	98	100	91*
101	100	98	100	95
FEMALES				
0	100	99	97	98
1	99	98	93*	89*
2	101	100	96*	94*
3	102	99	97*	96*
4	102	97*	96*	96*
5	102	97*	95*	97*
6	100	96*	93*	95*
9	99	95*	93*	95
13	100	95*	94*	94*
21	99	95*	94*	96
53	100	96	97	93*
81	90*	97	95	89
89	93	89	90	83*
101	104	105	91	87

4. Ophthalmological examination

Ophthalmological evaluations were performed on each rat prior to study initiation and during the 24-month of treatment on all survivors. No details were provided as to the methods used.

RESULTS

There were no apparent treatment-related lesions observed at any dose level.

5. Clinical Laboratory Investigations

Blood samples were collected from the same 20 rats/sex/group [where possible] no statement as to whether the rats were fasted prior to collection] after 3, 6, 12, 18, and 24 months of treatment [no information as to how the rats were chosen], and hematology and clinical chemistry evaluations were performed. The rats were anesthetized prior to blood collection via an orbital sinus [i.p. sodium pentobarbital]. No pre-test values were obtained.

a. Hematology: The CHECKED (X) parameters were examined.

X		X	
X	Hematocrit (HCT)	X	Leukocyte differential count♦
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count		Reticulocyte count
	Blood clotting measurements (Thromboplastin time) (Activated partial thromboplastin time) (partial thromboplastin time)	X	Red cell morphology
	Nucleated erythrocytes normoblasts		

♦ high dose and both control groups only [at 12 & 24 months]

RESULTS

None of the differences noted could be attributed to treatment.

b. Clinical Chemistry: The CHECKED (X) parameters were examined.

X	Electrolytes:	X	Other:
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorous	X	Cholesterol
X	Potassium	X	Globulin (calculated)
X	Sodium	X	Glucose
	Iron		Phospholipids
	Enzymes	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total Protein
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase (CPK)		Lipids, total
	Lactate dehydrogenase (LDH)		Triiodothyronine, total T_3
X	Serum alanine aminotransferase		
X	Serum aspartate aminotransferase		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase (GLDH)		
	Ornithine carbamyltransferase (OCT)		
	protein electrophoresis		
	Thyroxine, total T_4		
	Thyroid stimulating hormone (TSH)		

RESULTS

There were no changes in any of the monitored parameters that could be attributed to treatment.

c. Urinalysis: Prior to study initiation, freshly-voided urine samples were collected from all male and female rats [where possible]. Urine was collected from all males [where possible] and from 10 females/group at the end of the 3rd and 6th months of the study. At the end of the 12th, 18th, and 24th months of treatment, freshly-voided urine was collected from 10 rats/sex/group. At all collection times, the same rats scheduled for hematology/clinical chemistry analyses were used for the urinalysis determinations. The CHECKED (X) parameters were examined.

X		X	
X	Appearance (transparency)	X	Glucose
	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrite
X	Protein		Urobilinogen
	Leukocytes	X	Color

RESULTS

None of the findings were considered indicative of systemic toxicity. There was a statistically significant increase in specific gravity in both sexes at the 100 and 300 ppm dose levels after 3 and 6 months and in males at the 30 ppm dose level after 6 months. With continued treatment, this effect was not observed in either sex at any dose level. These increases are not unexpected in light of the decreased water intake observed at these time points.

Table 10. Urinalysis Findings

Parameter/Interval/Group	0 ppm	salt	30 ppm	100 ppm	300 ppm
Specific Gravity					
MALES					
pre-test	1.049	1.047	1.047	1.048	1.048
3-month	1.042	1.039	1.044	1.049*	1.058*
6-month	1.042	1.043	1.047*	1.050*	1.056*
12-month	1.046	1.045	1.046	1.050	1.048
18-month	1.047	1.041	1.044	1.050	1.062*
24-month	1.040	1.043	1.043	1.036	1.038
FEMALES					
pre-test	1.040	1.037	1.038	1.042	1.038
3-month	1.024	1.026	1.038	1.057*	1.053*
6-month	1.033	1.032	1.044	1.053*	1.049*
12-month	1.034	1.038	1.038	1.042	1.044
18-month	1.024	1.030	1.028	1.041*	1.033
24-month	1.029	1.028	1.033	1.045	1.044*

* $p<0.05$

6. Special Studies - Kidney Ultrasonography: The kidneys of all males on test were examined via ultrasound during the 6th and 12th months of the study to evaluate the incidence of dilated renal pelves, since a high incidence had been observed in a previous study.

RESULTS

There were no treatment-related effects observed at any dose level. At 6 months, 36 of the 443 rats [8.1%] examined displayed pelvic dilation [hydronephrosis] and 29 of the 432 rats [6.7%] examined at 12 months displayed dilation. Treated rats displayed the higher incidences at both time points, but there was no dose response [Table 7].

Table 7. Incidence of Pelvic Dilation [Hydronephrosis] (%)

Time/Group	tap H_2O	salt	30 ppm	100 ppm	300 ppm
6 months	8.1	5.6	8.9	10.3	7.8
12 months	3.6	5.7	6.8	9.4	8.0

7. Sacrifice and Pathology

At the interim sacrifices [12 and 18 months], 10 rats/sex/group were anesthetized, sacrificed by exsanguination, and necropsied. All survivors were sacrificed similarly at 24 months and subjected to a gross pathological examination. All organs, tissues, and body cavities

of the rats sacrificed on schedule, sacrificed in extremis, or dying on test were examined, and gross abnormalities were recorded. The brain, kidneys, liver, spleen, adrenals, and testes from each of the rats at scheduled sacrifice were weighed. The CHECKED (X) tissues were collected from all rats and preserved. Tissues listed below from 10 rats/sex/group at the interim sacrifices, all survivors [both sexes] at the 24-month sacrifice from both control groups and the high-dose group, and all rats that died or were sacrificed in extremis were examined microscopically. Only the kidneys, liver, lung, spleen, stomach, and gross lesions of the low- and mid-dose level rats were examined microscopically.

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
X	Tongue	X	Aorta	X	Brain♦
X	Salivary glands	X	Heart	X	Periph. nerve
X	Esophagus	X	Bone marrow♥	X	Spinal cord♦
X	Stomach	X	Lymph nodes♦	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes
X	Jejunum	X	Thymus	Glandular	
X	Ileum	Urogenital		X	Adrenal gland
X	Cecum	X	Kidneys*		Lacrimal gland
X	Colon	X	Urinary bladder*	X	Mammary gland
X	Rectum	X	Testes	X	Parathyroids
X	Liver	X	Epididymides	X	Thyroids
	Gall bladder	X	Prostate	Other	
X	Pancreas	X	Seminal vesicle	X	Bone (femur/sternum)
Respiratory		X	Ovaries	X	Muscle (skeletal)
X	Trachea	X	Uterus	X	Skin
X	Lung	X	Vagina	X	All gross lesions
	Nose	X	Cervix		and masses
	Pharynx	X	Coagulating gl.	X	Harderian glands
	Larynx				

♥ femur & sternum; ♦ mandibular & mesenteric;
♦ medulla/pons, cerebellar cortex, cerebral cortex
♦ cervical, mid-thoracic, lumbar

RESULTS

Treatment-related effects were observed in the stomach, which appear to be due to gastric irritation [see below].

a. Organ Weight: There were no apparent treatment-related effects on organ weight, although statistically significant differences were observed in some instances. Relative kidney weights were significantly increased in both sexes at the high-dose level at the 12-month interim sacrifice, but absolute kidneys were comparable among the groups in both sexes. In the high-dose females, the absolute kidney weight was greater than the control, while in the high-dose male it was decreased compared to the control. The author attributed the increase in relative kidney weight to the decreased body weight, which at termination [at 12 months] was 87% of the control in high-dose males and 96% of the control in high-dose females. Kidney weight findings are tabulated in Table 7, below. Decreased absolute adrenal weight [74% of control] was observed at the 18-month sacrifice in the high-dose males.

Table 7. Kidney Weight

Sex/Group/ Sacrifice Weight (g)	Absolute			Relative		
	12-month	18-month	24-month	12-month	18-month	24-month
MALES						
tap H_2O	4.734	4.454	4.678	6.862	6.250	8.154
salt	4.186(88)♦	4.577	4.332(93)	6.804	6.984(112)	6.587(81)
30 ppm	4.476(95)	4.674	4.657	6.847	7.227(116)	6.704(82)
100 ppm	4.462(94)	4.969(112)	4.686	6.705	7.143(114)	7.450(91)
300 ppm	4.629(98)	4.260(96)	4.356(93)	7.798*(114)	6.427(103)	7.033(86)
FEMALES						
tap H_2O	2.554	2.836	2.840	7.165	7.237	5.948
salt	2.638	3.171(112)	2.876	6.860	6.687(92)	6.099(103)
30 ppm	2.678(105)	2.627(93)	2.902	6.339(88)	7.193	6.225(105)
100 ppm	2.549	2.855	2.974(105)	7.182	7.525(104)	7.106(119)
300 ppm	2.732(107)	2.995(106)	2.866(101)	7.985*(111)	7.421(103)	7.021(118)

♦ (% of control); * $p<0.05$

b. Gross Pathology: No treatment-related gross lesions were reported for either sex in any tissue/organ other than in the stomach. There was an increased incidence of prominence of the limiting ridge and/or thickened nonglandular mucosa of the forestomach in both sexes at the mid- and high-dose levels. Additionally, the incidence of dark foci or areas in the gastric mucosa was increased at these two dose levels in both sexes [Table 8].

Table 8. Gross Pathology Findings

Lesion/Sex/Group	tap H_2O	salt	30 ppm	100 ppm	300 ppm
MALES N=90					
Stomach					
dark focus/area	8	9	12	16	24
prominent limiting ridge	2	2	1	4	25
depressed focus/area	6	8	5	11	13
white film, mucosa	6	9	7	8	14
thickened nonglandular mucosa	1	3	0	4	7
FEMALES N=80					
Stomach					
dark focus/area	5	5	3	13	11
prominent limiting ridge	1	1	1	5	26
depressed focus/area	3	5	7	6	8
white film, mucosa	3	7	4	4	10
thickened nonglandular mucosa	2	2	1	5	3

c. Microscopic Pathology: **Non-neoplastic Findings** - Hyperplasia and hyperkeratosis of the squamous mucosa of the forestomach were observed at the mid- and high-dose levels in both sexes, which correlated with the prominent limiting ridge and thickened nonglandular mucosa observed grossly. The incidence and severity occurred in a dose-related manner. Additionally, an increase in the incidence of necrosis of the granular mucosa was observed in both sexes, and an increase was observed in the incidence of ulcers of the forestomach, edema/inflammation of the glandular and forestomach submucosa in treated males [Table 9]. The incidence of glomerulonephrosis [progressive] in the kidneys and proliferation of the bile duct was increased in males at the high-dose level, and females at the mid- and high-dose levels displayed a dose-related trend, as well as significant increases [dose-related] in the incidences in vacuolation

in the adrenal gland and mineralization in the kidneys [Table 9].

Table 9. Microscopic Findings

Lesion, Sex, Group	tap H_2O	salt	30 ppm	100 ppm	300 ppm
MALES					
Stomach N=90					
hyperplasia/hyperkeratosis	8	9	4	16	45 ^{*S*}
necrosis, granular mucosa	11	10	12	12	26 ^{*S*}
ulcers, forestomach	1	2	4	5	7 ^{*T*}
edema/inflammation submucosa gl. ♦	5	9	8	10	13 ^{*T*}
edema/inflammation submucosa fs♥	4	4	7	10	13 ^{*S*}
Kidney N=90					
glomerulonephrosis, progressive	72	70	78	78	83 ^{*S*}
Liver N=90					
proliferation, bile duct	21	31	26	32	44 ^{*S*}
Parathyroids	88	88	49	45	89
hyperplasia	16	4	8	5	13 ^{*S}
FEMALES					
Stomach N=80					
hyperplasia/hyperkeratosis	5	5	9	17 ^{*S*}	38 ^{*S*}
necrosis, granular mucosa	3	9	6	11 ^{*T*}	13 ^{*T*}
ulcers, forestomach	2	2	2	2	2
edema/inflammation submucosa gl. ♦	1	3	2	9 ^{*T}	4
edema/inflammation submucosa fs♥	4	4	6	6	6
Kidney N=80					
mineralization, medulla, focal	0	3	4	5 ^{*T*}	6 ^{*T*}
Adrenal Gland N=	80	80	69	70	80
vacuolation, cortical, focal	6	11	4	13 ^{*T*}	14 ^{*T*}
Mammary Gland N=	80	80	66	63	79
hyperplasia	22	29	17	20	22
inflammation, granulomatous	0	0	0	1	1

♦ glandular; ♥ forestomach; ^{*S}=significant trend compared to both controls; ^{*T}=significant trend compared to tap water control; ^{*S} or ^{*T} significantly ↑ compared to both or tap H_2O control(s); ^{*S} significantly ↑ compared to salt control

There was no increase in the incidence of neoplasia in any organ/tissue in the males. Females displayed a significant positive trend in the survival adjusted cystadenoma incidence rates in the mammary gland compared to both control groups. When the combined mammary adenoma and cystadenoma incidence rates and the combined mammary adenoma, cystadenoma, and adenocarcinoma incidence rates are considered, either comparable or lower rates relative to the two control groups are observed. It is stated that these epithelial tumors should be combined because of their common origin.

Table 10. Neoplastic Findings in Mammary Gland

Tumor/Group	tap H_2O	salt	30 ppm	100 ppm	300 ppm
FEMALES					
Mammary Gland N=	80	80	66	63	79
adenocarcinoma	20/22♦	10/11	8/9	10/12	11
adenoma	2	5	6	1	2/3
cystadenoma	1	1	1/2	3	4 ^{*S}
adenoma + cystadenoma	3	6	7	4	6
adenoma + cystadenoma + adenocarcinoma	23(29)♦	16(20)	15(19)	14(18)	17(21)
fibroadenoma	23/38	17/27	16/22	14/27	20/35
sarcoma, histiocytic	0	0	1	1	0

♦ # rats with tumor/actual incidence of tumor [interpreted by reviewer to mean # of tumors]; ^{*S} significant positive trend compared with both controls; ♦ (%)

D. DISCUSSION

Exposure to Kathon® Biocide via the drinking water did not affect survival of either sex, and there were no signs of toxicity. Body weight and body-weight gains were decreased throughout the study in males at the high-dose level and in females at the mid- and high-dose levels, although during the first year of the study the mid-dose females displayed the greater decrease. During the second year, the decrease in the body-weight parameters was dose-related, and the high-dose females displayed a larger decrease in these parameters than the males. Neither the decrease in body weight [♂♂ 98%/♀♀ 97% of control] nor body-weight gain [♂♂ 96%/♀♀ 97% of control] for either sex showed statistical significance at 13 weeks. There was a dose-related decrease in water consumption by both sexes, which was significant throughout most of the study compared to the tap-water control groups, suggesting a palatability problem. High-dose males displayed a significant decrease [91-98% of control] in food consumption compared to the control groups throughout most of the study, and females at all dose levels displayed significant decreases in food intake but a dose response was not always evident. None of the differences observed in the hematology and clinical chemistry parameters monitored could be attributed to treatment. No ocular effects were observed in either sex, and no differences were found in the renal pelves of the males examined by ultrasonography. The specific gravity of the urine was increased in both sexes, which is not unexpected based on the decrease in water consumption. Organ weights were comparable among the groups of both sexes. With the exception of the stomach, none of the non-neoplastic lesions observed could be attributed to treatment. Glomerulonephritis is a common spontaneously-occurring lesion in male rats, and the rat strain used at the testing facility has a very high incidence. Since the severity was similar among the groups and within the reported incidence in 24-month studies, the increase was not considered to be of toxicological significance nor to be treatment related. Parathyroid hyperplasia was significantly increased in the high-dose males when compared only to the salt control group and is considered a compensatory and secondary response in chronic renal disease. In light of the slight increase in glomerulonephritis in the high-dose males, the two findings may be correlated. Other significant increases in non-neoplastic findings were considered common incidental findings and not of biological significance. In the stomach, treatment-related increases were observed in the incidence of thickness or prominence of the mucosa, frequently at the limiting ridge of the forestomach, which was due to hyperplasia and hyperkeratosis of the squamous mucosa. Necrosis of the glandular mucosa was increased at the high-dose level in males compared to both controls and at the mid- and high-dose levels in females compared to the salt control only. Other findings in the high-dose males included edema and inflammatory cell infiltration of the submucosa of the forestomach. There was no apparent increase in the incidence of neoplasms, although a significant increase in cystadenoma of the mammary gland in females was observed. When the incidence of these tumors is combined with the incidence of mammary adenomas and adenomas plus adenocarcinomas, no increase is evident. NOTE: In the cover letter [Transmittal Document] attached to the study, a Supplemental Report: Brown, WR (1994) Kathon® Biocide:24 Month Drinking Water Chronic/Oncogenic Study in Rats [Photomicrographs] is listed, which

apparently consists of 12 pages. The MRID # 431407-01 is written in next to this supplement, which is the MRID # for the study itself, which consists of 17 volumes. The photomicrographs were not included in the study report submitted to TB II [volumes 1-9], nor were they a part of the remaining volumes requested from Information Services Center. Although there is no need to submit these missing pages to TB II for review, for the record they should be located and included as part of MRID # 431407-01.

E. CONCLUSION

Under the conditions of the study, exposure of Crl:CD®BR rats [90 males/80 females/group to Kathon® Biocide via the drinking water at dose levels of 30 ppm [♂♂ 2.0/♀♀ 3.1 mg/kg], 100 [♂♂ 6.6/♀♀ 9.8 mg/kg], and 300 [♂♂ 17.2/♀♀ 25.7 mg/kg], or to tap water or 0.5% salt solution [$MgCl_2$ and $Mg(NO_3)_2$] vehicles for 24 months resulted in a dose-related decrease in drinking water consumption at all dose levels in both sexes throughout the study. Decreased body weight [95-98% of control]/body weight gain [87% of control at week one, from 91-98% thereafter] were observed in the high-dose males, although statistical significance was not always attained. Females showed an equivocal decrease throughout the first half of the study. During the second year, the high-dose females displayed an apparent treatment-related decrease in body weight/gain compared to the control females. High-dose males displayed a significant decrease [91-98% of control] in food consumption compared to the control groups throughout most of the study, and females at all dose levels displayed significant decreases in food intake but a dose response was not always evident. The hematology and clinical chemistry parameters monitored were comparable among the groups of both sexes, as were the organ weights and eyes. No differences were found in the renal pelves of the males examined by ultrasonography. The specific gravity of the urine was increased in both sexes, which is not unexpected based on water consumption. With the exception of the stomach, none of the gross and non-neoplastic lesions observed could be attributed to treatment. An increased incidence in hyperplasia and hyperkeratosis of the squamous mucosa of the stomach was observed at the mid- and high-dose levels in both sexes, which correlated with the finding of prominence of the limiting ridge and/or thickened nonglandular mucosa of the forestomach on gross examination. There was no treatment-related increase in the incidence of any tumor in either sex. **The NOEL can be set at 30 ppm [♂♂ 2.0/♀♀ 3.1 mg/kg/day], LEL at 100 ppm [♂♂ 6.6/♀♀ 9.8 mg/kg/day], based on the microscopic lesions [hyperplasia/hyperkeratosis in both sexes, necrosis of glandular mucosa in females] in the stomach.** This study is classified Core Minimum, and it satisfies the guideline requirement (83-5) for a combined chronic toxicity/ carcinogenicity study in a rodent.