

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH
SUMMARY OF TOXICOLOGY DATA

5-CHLORO-2-METHYL-4-ISOTHIAZOLIN-3-ONE and
2-METHYL-4-ISOTHIAZOLIN-3-ONE
(Kathon® 886 MW Biocide)

Chemical Code # 002038 and 002039, Tolerance # 50564 and 50566
SB 950 # 571 and 750

Original date: March 26, 2003

I. DATA GAP STATUS

Chronic/Onco, rat:	No data gap, acceptable study, no adverse effect
Chronic toxicity, dog:	Data gap, no study submitted.
Oncogenicity, mouse:	Data gap, inadequate study
Reproduction, rat:	No data gap, acceptable study, no adverse effect
Teratology, rat:	Data gap, inadequate study, no adverse effect indicated.
Teratology, rabbit:	No data gap, no adverse effects indicated.
Gene mutation:	No data gap, possible adverse effect indicated
Chromosome effects:	Data gap, no adverse mutagenic effect indicated
DNA damage:	No data gap, acceptable study, no adverse effects indicated.
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers through 164578 were examined.

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

File name: T030326

Original by: Kishiyama & Silva, 3/26/03

Note: (5-Chloro-2-Methyl-4-Isothiazolin-3-One and 2-Methyl-4-Isothiazolin-3-One) is a result of chemical reaction and not a blending of two active ingredients (no technical grade exists). File n930826 (letter of 5/6/92; in document 50564-065) provides additional details.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

** 50564 - 070 129142 "Kathon® Biocide: 24-Month Drinking Water Chronic/Oncogenic Study in Rats," (Quinn, D.L., O'Hara, G.P., Brown, W.R.; Rohm & Hass Toxicology Department, Report #: 90R-149; 1/24/94). Kathon® Biocide (14.2% ai) was administered in drinking water to Crl:CD®BR rats (90 males & 80 females/dose) at 0 (tap water), 0 (inorganic 'salt'), 30, 100 or 300 ppm (equivalent in M: 2.0, 6.6 & 17.2 mg/kg; F: 3.1, 9.8 & 25.7 mg/kg) for 24 months. Water consumption decrease of 0-22%, 3-30% and 15-40% for the respective low, medium and high dose groups was attributed to unpalatability of the active ingredient in Kathon. Chronic NOEL = 30 ppm (There was decreased water consumption at \geq 100 ppm and, food consumption and body weight at 300 ppm in both sexes. At 300 ppm, there was an increased incidence in thickened limiting ridge of the forestomach, due to hyperplasia/hyperkeratosis of the squamous mucosa. Both sexes at \geq 100 ppm had an increased incidence in dark foci or areas in the gastric mucosa. Males at \geq 100 ppm had an increased incidence in edema and inflammation of the submucosa of the forestomach. Males at 300 ppm had an increase in the incidence of focal necrosis of the superficial glandular mucosa.) ONCO NOEL = 300 ppm (There were no treatment-related oncogenic effects at any dose.) No adverse effect. Acceptable. (Kishiyama & Silva, 2/11/03).

CHRONIC TOXICITY, RAT

See Combined chronic/oncogenicity, rat study 070 129142 (above).

Subchronic Studies:

016 036810 "Kathon 886 NAR Three month Rat Drinking Water Study and One Generation Reproduction Study. Rohm & Haas Toxicology Department, Report 81R-162 9/9/82. COBS-CD (SD)BR rats (25/sex/dose) were exposed to compound at 0, 25, 75 and 225 ppm in drinking water based on a.i. of 15.5% (lot SW81-0138). Ten males and 10 females from each group were treated for 15 weeks and then mated to produce one generation of pups. No adverse effects were reported (except stomach erosion, glandular mucosa, lesions of mild magnitude). UNACCEPTABLE. Not upgradeable (too few animals in reproduction pilot study to address product safety). C. Aldous, 1/2/86). The animals in the subchronic toxicity phase were treated for 13 weeks. Blood samples were taken weeks 4 and 13 from 10/sex/dose for hematology (most parameters) and clinical chemistry (acceptable parameters). Histopathology was conducted at termination for both control groups (water and ion control) and high dose group. Stomachs were examined for all groups. Only the stomach showed treatment-related changes at 225 ppm (16.3 mg/kg: Males & 24.7 mg/kg: Females). Stomach findings: foci of erosion of epithelium (7/15 males, 3/15 females versus 0/15 in all controls), focal partial loss of surface mucous-same incidences, same animals. The study is acceptable as a subchronic study. (Amended 3/18/01, Gee).

EPA RED: NOEL = 75 ppm (6.3 mg/kg/day (M) 10.8 mg/kg/day (F), based on stomach changes.

** 50564 - 063 115311 "Kathon® 886 MMPA Process 13-Week Inhalation Toxicity Study in Rats," (Hagan, J.V., Baldwin, R.C.; Rohm and Haas Toxicology Department, Spring House, PA; Report #: 82R-245; Protocol #: 82P-093; 8/19/83; revised 12/10/84). Kathon 886 MMPA Process (11%RH-651, 3% RH-573, 16.5% Mg(NO₃)₂ in aqueous solution) was used in a whole body inhalation exposure on Crl:CD(SD)BR rats (16/sex/dose) at 0, 0.34, 1.15 and 2.64 mg a.i./m³ (6 hours/day, 5 days/week) for 13 consecutive weeks. Inhalation NOEL = 0.34 mg/m³ (Eosinophilic droplets in the anterior respiratory mucosa increased in both sexes at 2.64 mg/m³. Rhinitis incidence was increased in all treated groups for males and for females at \geq 1.15 mg/m³. The hyperplasia of the squamous epithelium, considered

to be secondary to the nasal irritation, was observed at 2.64 mg/m³, primarily in females.) Systemic NOEL = 1.15 mg/m³ (Body weights were statistically significantly lower in males weeks 1 - 12 at 2.64 mg/m³. Body weight gain was lower in both sexes at 2.64 mg/m³. week of dosing). Total protein was statistically significantly decreased in females and this effect was reported to be treatment related. The decreases in absolute spleen weights in males were treatment-related, dose related and statistically significant.) Acceptable, no adverse effect. (Kishiyama & Silva, 2/3/03)

CHRONIC TOXICITY, DOG

No study submitted

ONCOGENICITY, MOUSE

015 036809 "Kathon CG 30-Month Dermal Carcinogenesis Study in Male Mice," (Rohm & Haas Toxicology Department, Report #81R-288, 1/14/83). Kathon CG technical (5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one), 400 ppm a.i. in water. UNACCEPTABLE. Not upgradeable (incomplete, no NOEL, one dose level, too few animals, too much autolysis of tissue, etc.). (C. Aldous, 12/13/85).

REPRODUCTION, RAT

Subchronic Studies:

016 036811 "Kathon 886 NAR Three month Rat Drinking Water Study and One Generation Reproduction Study," (Rohm & Haas Toxicology Department, Report 81R-162; 9/9/82). COBS-CD (SD)BR rats were exposed to compound at 0, 25, 75 and 225 ppm in drinking water based on an a.i. of 15.5% (lot #: SW81-0138). Ten males and 10 females from each group were treated for 15 weeks and then mated to produce one generation of pups. No adverse effects were reported (except stomach erosion, glandular mucosa, lesions of mild magnitude). UNACCEPTABLE. Not upgradeable (too few animals in reproduction pilot study to address product safety). C. Aldous, 1/2/86).

Definitive Study:

** 50564 -065 164578 "Kathon[®] 886F Biocide: Two-Generation Reproductive Toxicity Study in Rats," (Robison, P., Craig, L.P., Danberry, T.L.; Rohm and Haas Company Toxicology Department, Spring House, PA; Protocol #: 96P-189; Report #: 96R-189; 8/7/98). Kathon[®] 886F Biocide (14.8% a.i.) was administered in drinking water to CrI:CD[®]BR rats (26/sex/dose) at 0 (drinking water), 0 (salt control), 30, 100 and 300 ppm from P1 adult generation through weaning of F2 pups (21 days post-partum). Systemic NOEL = 30 ppm (There was a statistically significant decrease in P1 male pre-mating body weights weeks 2 - 4 and cumulative body weight gain weeks 0 - 5 at 300 ppm. P1 males had statistically significantly decreased terminal body weight at 300 ppm. P2 females had decreased cumulative body weight gain days 0 - 21 of lactation. There was statistically significantly decrease food consumption at ≥ 100 ppm in both sexes of P1 adults at pre-mating. Both sexes of P1 and P2 adults had statistically significantly decreased water consumption throughout pre-mating at ≥ 100 ppm. P1 females had decreased water consumption at ≥ 100 ppm during gestation and lactation. P2 females had decreased water consumption during gestation and LD 7 - 14 at ≥ 100 ppm. There was an increased incidence in erosions, edema/inflammation, hyperplasia and hyperkeratosis of the stomach in P1 and P2 adults at ≥ 100 ppm. Both sexes of P1 adult kidney weights and P1 female kidney weights were increased and P1 males had decreased relative liver weights at 300 ppm at termination. P2 males had statistically significantly increased trend and weights for relative adrenal, thymus, seminal vesicle + coagulating gland and kidney weights at termination. P2 females had increased relative kidney and thymus weights at ≥ 100 ppm at termination.) Reproductive NOEL > 300 ppm

(There were no treatment-related reproductive effects at any dose.) Pup NOEL = 30 ppm (F1 pups of both sexes had statistically significantly decreased terminal body weights and males had decreased absolute brain weights at 300 ppm. Female F1 pups at 300 ppm had a trend for increased relative brain weight and statistically significantly increased relative thymus weights at ≥ 100 ppm.) Acceptable. No adverse effect. M. Silva, 2/19/03

TERATOLOGY, RAT

029 036823 "Teratology Study in Rats: Kathon 886," (Hazleton. 9/25/80). Kathon 886 (5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one, grade and purity not given; Lot #: 78/4435) was administered at 0, 10, 30, 100 mg/kg/day. No developmental toxicity observed (NOEL = 100 mg/kg/day). UNACCEPTABLE. Upgradeable (dose levels need justification, individual data required for clinical observation; other clarification needed). (C. Aldous, 1/2/86). EPA RED: Maternal NOEL = 30 mg/kg/day (decreased body weight gain and mortality).

TERATOLOGY, RABBIT

** 50564 - 065 115321 "Kathon Biocide: Oral (Gavage) Developmental Toxicity Study in Rabbits Protocol No. 91P-074 Report No. 91R-074," (Thomas, T.L., Solomon, H.M., O'Hara, G.P.; Rohm and Haas Company Toxicology Department, Spring House, PA; 4/28/92). Kathon 886 MW Biocide (13.4% a.i.; 75% 5-chloro-2-methyl-4-isothiazolin-3-one & 25% 2-methyl-4-isothiazolin-3-one) was administered by gavage to mated New Zealand white rabbits (16/dose) at 0 (tap water), 0.5, 2, 8 or 20 mg/kg during gestation days 7 through 19. Nominal Maternal NOEL = 2 mg/kg (A statistically significant increase in mortality occurred at 20 mg/kg. Clinical observations increased at ≥ 8 mg/ml, primarily during the treatment period (GD 7 - 19). At 8 mg/kg/day there was a statistically significantly lower body weight gain and food consumption. Necropsy findings showed a statistically significant increase in stomach irritation at ≥ 8 mg/kg.) Developmental NOEL = 8 mg/kg (No treatment-related developmental effects were observed in the study.) No adverse effect. ACCEPTABLE. (Kishiyama & Silva, 2/7/03)

027 036821 "Teratology Study in Rabbits," (IRDC, 7/8/77). Kathon 886, technical grade assumed (5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one; lot #: 6-2367, purity not stated) was administered at 0, 1.5, 4.4, and 13.3 mg/kg/day. All doses caused excessive deaths - No NOEL; insufficient data to evaluate developmental toxicity potential. UNACCEPTABLE. Not upgradeable (too few survivors to permit assessment of teratological potential). (C. Aldous. 1/2/85). NOTE: Since the pilot study doses were based on a 14% a.i., presumably these doses were also adjusted (see Volume/record #: 028/036822).

GENE MUTATION

50564 - 064 115316 "Kathon 886: *Salmonella typhimurium* Gene Mutation Assay," (Sames, J.L., Frank, J.P.; Rohm and Haas Company Toxicology Department, Spring House, PA; Report No. 90R-0142; 7/24/90). Kathon 886 (14% a.i. = 5-chloro-2-methyl-4-isothiazolin-3-one + 2-methyl-4-isothiazolin-3-one) was evaluated for mutagenic potential at 0 (water), 1.6, 3, 5, 6, 9, 10, 16, 20, 30 and 60 : g a.i./plate, using *Salmonella typhimurium* strains TA98 and TA1537 (+/- S9 metabolic activation). Kathon 886 without S-9 Mix was toxic at all dose levels. An additional assay was performed where S9 activation was preincubated with 3, 6, 10, 20, 30 or 60 ug/plate Kathon 886 for 2 hours (room temperature) prior to addition of bacteria (TA98 & TA1537) and agar. **Possible adverse effect indicated:** Kathon 886 induced mutagenic activity when S9 metabolic activation was used with TA98 (≥ 20 ug/plate) and TA1535 (≥ 6 ug/plate). With S9 and a 2 hour preincubation, there was a statistically significant increase in revertant colonies with TA1537 at 60 ug/plate Kathon 886. Assessment of mutagenic potential of Kathon 886 without metabolic activation was not possible due to

excessive toxicity at all dose levels. The Positive controls were functional. Not acceptable and not upgradeable (Too few strains of bacteria tested, in addition to other deficiencies). (Kishiyama & Silva, 2/3/03).

** 024 036818 "Drosophila Sex-Linked Recessive Lethal Test on Kathon Biocide," (Zoology Department, 17.2% a.i.; lot #: SW-8061; University of Wisconsin, 12/29/82). Male flies were fed 300 (52 ug/ml a.i.) or 500 ppm or injected with 1500 ppm (258 ug/ml a.i.); mated 3, 2, and 2 days 1:3: test material caused mortality and sterility, but no sex-linked recessive lethals. UNACCEPTABLE. Upgradeable (Remsen, 1/10/86). NOTE: Test article was equivalent to technical. Study upgraded (Gee, 3/18/03).

** 023 036817 "Mutagenicity Evaluation of TD-81-155 in the Mouse Lymphoma forward Mutation Assay," (Litton Bionetics, December 1981). Mouse lymphoma L51784 TK/- with and without rat liver activation at 0 - 2 nl/ml (-S9) or 0 - 30 nl/ml (+S9), **increased mutation frequency with and without activation**. ACCEPTABLE. (Remsen, 1/21/86).

019 036813 "Kathon 886; Microbial Mutagen Test," (Rohm & Haas, Report 81R-96; 5/21/81). *Salmonella typhimurium* TA1535, TA1537, TA98 and TA100 ± S9, was used at 0.0005 to 0.005 : l/plate (Toxic at higher amounts; lot #: 6-2368; no purity stated); no repeat trial, no individual plate values; **marginal increase rate in TA100 (-S9) only**. UNACCEPTABLE. Upgradeable (incomplete). (Remsen, 1/8/86).

018 036812 "Mutagenicity Evaluation of Kathon 886," (Litton Bionetics; 7/12/76). Kathon 886 (lot #: SW/0098; purity not stated) was used on *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100, with and without rat liver activation, at 0.00005 - 0.1 : g [: l] per plate; single value per concentration only; repeat with **TA100 at high concentration only; marginal positive response**. UNACCEPTABLE. Upgradeable. (Remsen, 1/9/86).

020 036814 "Kathon 886 NAR Process; Microbial Mutagen Test," (Rohm & Haas; Report 81R-97; 5/21/81). *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100, with and without rat liver activation, were treated with Kathon 886 at 0.00005, 0.00075, 0.0010, 0.0025 and 0.005 : l/plate (lot #: 81-7211, 15%, tested as is); cytotoxic -S9 at high concentration; **increase reversion rate -S9 with TA100**; mean colony count only; no repeat trial - see #'s 36813 and 36813. UNACCEPTABLE. Upgradeable. (Remsen, 1/9/86).

CHROMOSOME EFFECTS

50564 - 064 115319 "Acute Test for Chemical Induction of Chromosome Aberration in Mouse Bone Marrow Cells *In Vivo*," (Gudi, R.; SITEK Research Laboratories, Rockville, MD; SITEK Study #: 0159-1541; R & H Report #: 90RC -169; 4/29/91). Kathon 886 (13.9% ai; 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one) was administered in a single gavage to CD-1 mice (5 - 7/sex/dose/time point) at 4, 20 or 40 mg/kg. Five additional mice/sex at 0 and 40 mg/kg were monitored for clinical signs and body weight. Animals were terminated at 6, 24 and 48 hours and bone marrow was examined. Mortality at 48 hours was excessive and precluded performing the chromosome aberration test at 40 mg/kg. Low and mid-dose did not statistically significantly increase chromosome aberrations by 6, 24 and 48 hours post-dosing. Unacceptable but possibly upgradeable with submission of information about stability of a.i. and of dosing material. (Kishiyama & Silva, 2/5/03)

026 036820 "*In vitro* Chromosomal Aberration with Kathon CG," (Tochigi Labs, Kao Corporation. 1982). Kathon CG at 1.5% a.i., Chinese Hamster lung fibroblasts exposed 24 or 48 hours without activation to 0, 0.03125, 0.0625, 0.125, 0.25 or 0.5 : g Kathon (not a.i.) per ml. >0.5: g/ml was toxic, no increase in aberrations were reported. UNACCEPTABLE. Not upgradeable. Remsen, 1/8/86).

017 036867 "Cytogenetics Studies Compound RH 886 T," (Litton Bionetics, 1973).

5-chloro-2-methyl-4- isothiazolin-3-one (no lot number or purity or information on whether doses were corrected) was used on 5 male rats per group at 0.5, 5.0, or 50 mg/kg for 5 days orally (not specified whether by gavage or in feed); 3 controls and 5 given TEM ip.; apparently killed 5 days after last treatment; no increase in aberrations reported. UNACCEPTABLE. Not upgradeable. (Remsen, 1/8/86).

025 036819 "Micronucleus Test on Kathon 886," (Takeda Chemical Industries, June 1983). Kathon (16% a.i., Lot #: 10-2-81), Five males per group given a single oral gavage dose of 3, 9 or 30 mg/kg (calculated to be a.i.) or 5 doses of 6 mg/kg. After a single dose, animals were killed 30 hours later; after 5 doses, after 6 hour period; bone marrow was examined for micronucleated erythrocytes; no increase with treatment reported. (Remsen, 1/8/86).

022 036816 "Kathon 886 (NAR) Cytogenetic Study in Mice," (Rohm & Hass Toxicology Department, 2/20/82). Eight males per group were given 10, 40 or 100 mg/kg by oral gavage as single dose and killed 6, 24, or 48 hours later or 5 daily doses and killed 6 hours after the last dose (lot #: SW81-7211, 15%) doses were uncorrected for purity; TEM control; no adverse effect on chromosome reported; no clinical effects reported. UNACCEPTABLE. Not upgradeable (no males; no MTD). (Remsen, 1/8/86).

DNA DAMAGE

50564 - 064 115318 "Test for Chemical Induction of Unscheduled DNA Synthesis in Rat Primary Hepatocyte Cultures by Autoradiography," (Pant, K.J., SITEK Research Laboratories, SITEK Study #: 0159 - 5100; R & H report #: 90RC-168; 4/24/91). Kathon 886 (13.9% ai) was used on primary rat hepatocytes *in vitro* at 0.03475 to 3.475 : g ai/ml and 0.0371 to 2.224 : g/ ai/ml in Trial 1 and Trial 2, respectively, to evaluate the potential to induce unscheduled DNA synthesis. No increase in net nuclear grain counts was observed at any dose. The positive controls functioned as expected. Currently, this study is not acceptable but is possibly upgradeable with submission of individual data and characterization of test material. No adverse effect indicated. (Kishiyama & Silva, 2/4/03)

** 021 036815 "Kathon 886 Mammalian Cell Transformation Test," (Rohm & Haas Toxicology Department. 6/29/81). *In Vitro* transformation with C3H 10T ½ cells exposed for 24 hours to 0, 0.05, 0.1, 0.3, 0.5, 0.6 and 0.8 nl/ml of Kathon (15% a.i., lot #: SW-81-7211); fixed and stained after 6 weeks and scored for Types I, II, & III foci, no increased transformation. ACCEPTABLE. (Remsen, 1/10/86).

NEUROTOXICITY

Not required at this time.