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

OCT 17 2007

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: **Toxicology Study Reviews of Kathon™ (Kathon 886 composed of Kathon 651 and Kathon 573) and their Kathon Metabolite; Othilinone (Kathon RH-893) and a Metabolite of Kathon 287**

EPA Reg. No.: 707-GNT-Kathon 287 WT Wood Preservative
DP Barcode: D341456
PC 128101

To: Marshall Swindell, PM 33
PM Team Reviewer, Demison Fuller
Regulatory Management Branch
Antimicrobials Division [7510C]

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Risk Assessment and Science Support Branch (RASSB) Antimicrobials Division (AD)[7510C]

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and

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Uses: Antimicrobial, non-food use

Sponsor: Rohm and Haas, Inc. Springhouse, PA

BACKGROUND: The following toxicology executive summaries [and DERs] on various Kathons and Kathon metabolites have been complied to fulfill bridging requirements on the Kathon RH-886 vs. RH 287.

I. Octhilinone (Octyl-isothiazolone, 2-n-octyl-4-isothiazolin-3-one), Kathon 893

1. **Subchronic Oral Toxicity in Dogs (diet);** MRID 47154016

EXECUTIVE SUMMARY: In this subchronic oral toxicity study (MRID 47154016), an initial study of 4 beagle dogs/sex/group were administered Acticide® OIT (95.9% a.i.; Batch #K3946 511) in the diet at nominal concentrations of 0, 100, 300, or 1000 ppm. Approximately 58 weeks later, an additional 4 dogs/sex/group were added to the study and were fed test diets at concentrations of 0 (concurrent control group), 3000 or 6000 ppm. The dogs fed the 6000 ppm diets had decreased food consumption and body weights for Days 1-8 and were thus placed on control diet supplemented with canned food from Days 9-12. These animals: received 4500 ppm diets from Days 14-21; were then inadvertently fed the 6000 ppm diets from Days 22-30; and finally remained on 4500 ppm diets from Day 31 until study termination.

There were no effects of treatment on clinical signs, hematology, clinical chemistry, urinalysis, ophthalmoscopy, organ weights, gross pathology, or histopathology.

At 4500 ppm, one female (#54) was euthanized on Day 30. Inanition was considered to be the cause of this dog's poor clinical condition, and it was described as emaciated at necropsy. All other animals survived until scheduled termination.

Poor palatability of the test diet in the dogs initially fed 6000 ppm was evident by increased food scatter and decreased food consumption, with concomitant decreases in body weight gains and body weights. The fact that the dogs in the high dose group exhibited food aversion without systemic toxicity indicates that the test material should have been administered in capsules ands in the diet.

In the dogs initially fed the 6000 ppm diets (hereafter referred to the 4500 ppm group), body weights were decreased during the first week of treatment (decr. 9-11% on Day 8). Despite being switched to an untreated diet supplemented with canned dog food for one week and then placed on a 4500 ppm diet, the body weights of the females at this dose remained decreased by 12% ($p \leq 0.05$) on Day 22. These animals were then inadvertently fed the 6000 ppm diets from Days 22-30 and finally remained on 4500 ppm diets from Day 31 until study termination.

Absolute body weight gains (calculated by the reviewers) were decreased at 4500 ppm for Days 1-8 in the males (-0.5 kg treated vs 0.0 kg controls) and females (-0.6 kg treated vs 0.0 kg controls). Absolute body weight gains for the overall (Days 1-92) study remained lower in the females at 4500 ppm (-0.6 kg) compared to controls (0.4 kg).

Percent (of pre-test) body weight gains were decreased ($p \leq 0.05$) in the 4500 ppm males on Days 8 and 29. In the females, percent body weight gains were decreased at 4500 ppm on Days 8, 15, 22, 29, 50, 85, and 92. Percent body weight gains were decreased ($p \leq 0.05$) at 3000 ppm intermittently toward the end of the study.

Absolute food consumption (g/animal/day) was decreased by 28-58% ($p \leq 0.05$) for Days 1-8, 15-22, 22-29, and 85-91 in the 4500 ppm males, resulting in an overall (grand mean) decrease of 16% compared to controls. In the females, absolute food consumption was

decreased ($p \leq 0.05$) by 25-34% for Days 1-8 and 8-15 at 3000 ppm. Additionally in the 4500 ppm females, decreases of 13-65% were observed for Days 1-8, 8-15, 15-22, 22-29, 71-78, and 78-85, resulting in an overall decrease of 19% compared to controls.

Relative (to body weight) food consumption was decreased by 55% ($p \leq 0.01$) for Days 1-8 in the 4500 ppm males, resulting in a decrease of 12% for the overall study. In the females, relative food consumption was decreased ($p \leq 0.05$, except as noted) at 3000 and 4500 ppm for Days 1-8 (decr. 33-61%) and Days 8-15 (decr. 17-19%; NS at 4500 ppm), and remained decreased at 4500 ppm for Days 15-22 (decr. 42%; $p \leq 0.05$).

The LOAEL was not observed. The NOAEL is 4500 ppm (equivalent to 135.8/160.0 mg/kg/day in males/females).

This study is classified **UNACCEPTABLE - GUIDELINE** and does not satisfy the guideline requirements (OPPTS 870.3150; OECD 409) for a subchronic oral toxicity study in dogs because a LOAEL was not observed and the animals were not tested to the limit dose of 1000 mg/kg/day.

II. 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-3(2H) isothiazoline (3:1 ratio). The combination formulation is also called RH -886

3. ***In vivo* Mammalian Cytogenetics – Chromosomal Aberration Test in Bone Marrow of Mice**; MRID 47154022

EXECUTIVE SUMMARY: In a CD-1 mouse bone marrow chromosome aberration assay (MRID 47154022), 8 male mice/dose/sampling period were exposed by oral intubation to a single dose of 0, 10, 40, and 100 mg/kg Kathon 886 (NAR) (acute regime). An additional 8 male mice/dose were exposed by oral intubation to a single dose for five days (subacute regime). Bone marrow cells were harvested at 6, 24, or 48 hours post-treatment (acute regimen), or 6 hours post-treatment (subacute regimen). The vehicle used was distilled water. Triethylene melamine (0.3 mg/kg), administered by intraperitoneal injection, served as the positive control. Bone marrow cells for the positive control treated group were harvested at 24 hours post-treatment.

There were no clinical signs of toxicity or mortality observed during the study for any test group. No significant increases in chromosomal aberrations were observed in animals treated with 100 mg/kg following both dosing regimens (acute and subacute) or in animals sacrificed 48 hours after being treated with 40 mg/kg (acute regimen). The positive control elicited a clear, positive response. **There was no evidence of chromosome aberration induced over background.**

This study is classified as **ACCEPTABLE - GUIDELINE** and satisfies the guideline requirement for the Test Guideline OPPTS 870.5385; OECD 475 for *in vivo* cytogenetic mutagenicity data.

4. ***In vitro* Mammalian Cytogenetics Chromosomal Aberration Assay;** MRID

47154023

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics chromosomal aberration assay (MRID 47154023), Chinese hamster lung fibroblast (CHL) cells were exposed to Kathon CG (1.5% a.i., Batch/Lot No.: not provided) at concentrations of 0, 0.03, 0.06, 0.125, 0.25, 0.50, 1, 2, 4, and 8 µg/mL without metabolic activation for 24 and 48 hours. Cells were harvested immediately after treatment and analyzed for chromosomal aberrations.

Kathon CG was tested beyond cytotoxic concentrations. Toxicity was observed at 1, 2, 4, and 8 µg/mL dose levels; however, chromosomal aberrations were not evaluated for these groups due to insufficient number of cells. Chromosomal aberrations were assessed for 0.03, 0.06, 0.125, 0.25 and 0.50 µg/mL dose levels and Kathon CG induced chromosome gaps, breaks, and exchanges; however, the aberrations did not exceed background levels. The percentage of aberrations ranged from 1–6% for 24 and 48 hours treatment periods and was comparable to the solvent controls (4%). Positive controls did induce the appropriate response. **There was no evidence of chromosome aberration induced over background.**

This study is classified as **UNACCEPTABLE - NONGUIDELINE** and does not satisfy the guideline requirement for the Test Guideline *in vitro* mammalian cytogenetics chromosomal aberration assay OPPTS 870.5375; OECD 473 for *in vitro* cytogenetic mutagenicity data. See Section III.B for the discussion on study deficiencies.

5. ***In Vivo*** **Mammalian Cytogenetics - Erythrocyte Micronucleus assay in Mice Bone Marrow;** MRID 47154024

EXECUTIVE SUMMARY: In a bone marrow micronucleus assay (MRID 47154024), 5 male Crj:CD-1 (ICR) mice were treated once orally with Kathon 886 (16% a.i., lot # 10-2-81) in distilled water at doses of 0, 18.8, 56.3, or 187.7 mg/kg bw (equivalent to 3, 9 and 30 mg/kg. bw a.i., respectively). Additionally, 5 male Crj:CD-1 (ICR) mice were treated daily, for five consecutive days, by oral gavage, with Kathon 886 (16% a.i., lot # 10-2-81) in distilled water a dose of 37.5 mg/kg bw. Bone marrow cells were harvested at 30 hours post-treatment, for animals receiving a single dose, and 6 hours post-treatment, for those animals receiving five consecutive doses. Endoxan was used as a positive control.

There were no signs of toxicity or mortality reported during the study. No statistically significant increases in the frequency of micronucleated polychromatic erythrocytes were observed in any treatment group when compared to controls. The positive control induced the appropriate response. **There was not a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.**

This study is classified as **ACCEPTABLE - GUIDELINE** and satisfies the guideline requirement for the Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

6. ***In Vitro* Mammalian Cells in Culture Gene Mutation Assay in C3H 10T1/2 Mouse Embryo Fibroblast Cells**; MRID 47154021

EXECUTIVE SUMMARY: In a mammalian cell transformation test (MRID 47154021), C3H 10T1/2 mouse embryo fibroblasts were seeded in cell plates, exposed to Kathon 886 (15% a.i., Lot No. SW-81-7211) at concentrations of 0, 0.05, 0.10, 0.30, 0.50, 0.60, or 0.80 nL/mL without metabolic activation for 24 hours. After subsequent incubation for six weeks, the plates were stained with 10% Giemsa and transformed foci (type I, II, and II) were scored by Quebec Colony Counter.

Kathon 886 was tested up to the toxicity limit as determined by the range finding assay. In the transformation test, no type III foci were observed in any of the 113 Kathon 886-treated plates. Only one type III transformed foci was observed in the untreated control plates. This transformation was considered to be spontaneous, and as this result was observed in the control plate, it was not considered treatment-related. Positive controls induced the appropriate response. **There was no evidence of cell transformation induced over background.**

This study is classified as **UNACCEPTABLE - NONGUIDELINE** and does not satisfy the guideline requirement for Test Guideline *In vitro* mammalian cell gene mutation

assay OPPTS 870.5300; OECD 476 because metabolic activation of the test material was not included.

7. **Dermal Carcinogenicity Study in Mice;** MRID 47154028

EXECUTIVE SUMMARY: In this carcinogenicity study (MRID 47154028), Kathon CG (1.5% a.i.; Lot No.: MH31:9E) in distilled water was administered 3 x each week by dermal application to a shaved 2 x 3 cm area of the skin to 40 male CD-1 mice at 400 ppm a.i. in a dose volume of 25 µL for up to 30 months. Kathon CG is a commercial formulation containing the active ingredients 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one as a 3:1 mixture. A negative control of distilled water and a positive control of 1000 ppm 3-methylcholanthrene were also tested as above on 40 male CD-1 mice/each. Clinical signs, body weights, and macroscopic pathology were reported for all animals. Limited microscopic pathology was also performed for the Kathon CG and negative control groups. Histopathological examination of the positive control group was limited to treated skin and gross lesions.

No treatment-related effect was noted on body weights or body weight gains or on the incidence of macroscopic lesions.

A slight increase in mortality was observed in the Kathon CG group relative to control after 12 months of treatment. Survival rate after 2 years was 32.5% in the Kathon CG group vs 67.5% in the negative control group. The difference in survival rate at 30 months was less than at 24 months, possibly due to the increase in age-related death in the control group. Overall survival distributions, utilizing time to death or study termination in months for each animal, was significantly different ($p \leq 0.05$) as determined by the generalized Wilcoxon test, but not by the log-rank test. Additionally, a difference in survival at 30 months was not detected by the chi-square test. Systemic toxicity was not corroborated by other findings in this test; however, many parameters examined under the current guidelines were not evaluated. Consequently, the effect of 400 ppm Kathon CG on survival was considered equivocal.

In the Kathon CG group, a treatment-related increased incidence of eschar, dessication, or flaking skin and brown stain was noted at the application site (9-10/40 treated vs 0-1/40 controls). The incidences of the following signs were increased, but it is not clear if these signs were due to treatment: pale, white, opaque eye(s), ataxia, and convulsive-like behavior (7/40 treated vs 1-2/40 controls). The Sponsor stated that pale, white, opaque eye(s) were generally seen prior to death or the later stages of the study and considered this finding to be age-related. The Sponsor also stated that ataxia and convulsive-like behavior were generally observed just prior to death.

In the Kathon CG-treated skin, incidence and lesion severity was increased for very slight to moderate dermal collagen (30/39 treated vs 26/39 negative controls) and very slight to moderate epidermal hyperplasia (28/39 vs 3/39). Increased incidences of the following lesions were also noted in the Kathon CG-treated skin (# affected/39 in treated vs negative controls): (i) focal/multifocal epidermal necrosis (12 vs 0); (ii) eschar (5 vs 0); (iii) hyperkeratosis (7 vs 0); and (iv) dermal inflammation (6 vs 0). Systemically, an increased incidence of focal/multifocal hepatic necrosis was noted (9/40 treated vs 2/40 negative controls), as was a slight increase in the incidence of fundic mucosa dilated glands in the stomach (21/40 treated vs 16/40 negative controls). Without historical control data, it is difficult to conclude that these isolated systemic findings are treatment-related.

In the 3-methylcholanthrene positive control group, 38/40 mice had died by Month 12, and the remaining mice died by Month 16. Pale, white, opaque eye(s) (19/40 positive controls vs 2/40 negative controls) and ataxia (8/40 vs 2/40) were noted. Body weights of were slightly increased (↑22% at Day 309 when 11 mice remained alive), which may have been a result of the tumor-burden. Increased incidences (# affected/40 in positive control vs negative control groups) of enlarged livers (4 vs 0), white nodules in lungs (15 vs 6), enlarged spleen (28 vs 3), and masses at the application site (40 vs 0) were observed. An increased incidence of myeloid hyperplasia in the spleen was noted (11/29 treated vs 0/40 controls).

The incidence of each neoplastic lesion in the Kathon CG-treated group was similar to the negative controls; however, a <u>total</u> of 4 neoplastic lesions were noted in the treated skin compared to 1 neoplastic lesion in the negative control. As evidence of a carcinogen

is typically provided by a conclusive increase in incidence of a particular neoplastic lesion, Kathon CG is tentatively not considered a carcinogen.

The positive control, 3-methylcholathrene, induced squamous cell carcinoma in all treated animals (40/40 treated vs 0/39 negative controls). Metastatic squamous cell carcinoma was noted in the lungs (19/22 treated vs 0/40 negative controls). Myelogenous leukemia was noted in the lungs (14/22) and spleen (17/29) of the positive controls (vs 0/40 negative controls).

At the doses tested, there was no treatment-related increase in tumor incidence when compared to controls. Dosing was considered adequate based on increased mortality.

This study is classified as **UNACCEPTABLE - NONGUIDELINE**. Stability data for the dosing preparations were not provided; the study is **upgradeable** pending submission of the required data. The study is unacceptable due to the fact that one dose was used; the dermal exposure site was not occluded, washed, or protected from oral exposure via grooming or licking; several toxicological parameters were not analyzed.

III. 5-chloro-2-methyl-3(2H) isothiazolone (RH-651)

8. **Metabolism** – Rat; MRID 47154007

EXECUTIVE SUMMARY: In a 96-hour metabolism study (MRID 47154007), ^{14}C-RH-651 (Lot #1018.0013, 99.95% radiochemical purity) dissolved in water was administered by gavage at a dose of 3.75 or 22.5 mg/kg to male rats and at a dose of 3.75 or 11.25 mg/kg to female rats (4 animals/sex/dose). The dosing solutions also contained Kathon 886F (14.11% a.i.) composed of RH-573 (51.4% a.i.) and RH-651 in an approximate 3:1 ratio.

In the low-dose group, 85.73%-87.16% of the radioactive dose was recovered by 96 hours post-dosing, with 29.75%-30.75% found in the urine, 47.27%-48.77% in the feces, 0.93%-1.44% in selected tissues, and 6.29%-7.71% in cage rinse. In the high-dose group, 94.56%-95.60% of the radioactive dose was recovered, with 38.57%-43.03% found in the urine, 43.87%-45.90% in the feces, 3.94%-4.72% in selected tissues, and 4.76%-5.38% in cage rinse. Whole blood (not including plasma) had the highest percent radioactivity in tissues (0.67%-1.09% for the low-dose group, 3.41%-4.11% for the high-dose group). Each of the other tissues contained less than 1% of the dose. Most of the radioactive dose was eliminated within 24 hours post-dosing (77.20%- 86.68%).

A total of approximately 29 metabolites of RH-651 were detected in urine and feces samples collected 0-48 hours post-dosing. The main metabolite in the urine, M1A, contained 15.35%-18.19% of the radioactive dose. The main component (approximately 94.38%) of M1A, M1A-1, was identified as N-methylmalonamic acid. The main metabolite in the feces, designated as M15, contained 26.38%-32.54% of the radioactive dose. Metabolite M1B, found in the feces, contained 5.19%-9.62% of the dose. Each of

the other metabolites comprised <5% of the dose. Structures were determined for M1A (component M1A-1), M1B (components M1B-1 and M1B-3), M2, M7, M13, M15, M20, and M24 (component M24-A). A metabolic pathway was proposed for RH-651 in the rat.

This metabolism study in the rat is classified **ACCEPTABLE - NONGUIDELINE** and does not satisfy the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in the rat. The study is upgradable if justification is provided for not collecting expired air samples, or urine samples at 6 and 12 hours post-dosing and/or if these requirements are waived. **The absence of these data does not significantly affect the results of the study.**

9. **Metabolism** – Rat; MRID 47154008

EXECUTIVE SUMMARY: In a 24-hour metabolism study (MRID 47154008), [^{14}C]-RH-651, (Lot number 1018.0012) was administered to bile duct cannulated Sprague Dawley rat. A single oral dose of 3.75 mg/kg was administered to a group of 3 female rats with excretion collections for 24 hours. During the 24 hours an average of 4.74% of the administered dose was excreted in bile. The dose recovered as measured by the amount of radioactivity in the urine, cage rinse, and feces were 44.09%, 7.96%, and 24.72%, respectively. Total recovery during the 24-hour period in bile, urine, cage rinse, and feces averaged 81.5%. At least 20 metabolites were observed in urine and feces. Among these N-methyl-malonamic acid (M1A-1) was detected in urine as the major metabolite for RH-651 (26.3% of dose). Parent compound was not detected in either urine or feces sample.

This metabolism study in the bile duct cannulated rats is classified **ACCEPTABLE-GUIDELINE** for a tier 2 metabolism study and satisfies only the guideline requirement for a metabolism study characterizing metabolites [OPPTS 870.7485, OECD 417] in rats.

10. **Tissue Distribution in Mice**; MRID 47154017

EXECUTIVE SUMMARY: In a non-guideline tissue distribution study (MRID 47154017), [4,5-^{14}C]-RH-651 (Lot # 1018.0013; radiochemical purity 99.9%) in NANOPure® water was administered to 15 CD-1 mice/sex at a dose level of 22.5 mg base-eq./kg (containing 7.5 mg/kg RH-573) by a single oral gavage at a dose volume of 10 mL/kg. At 1, 3, 6, 24, and 48 h post-dosing, three mice/sex were killed, and blood, plasma, bone, bone marrow, and liver were collected and analyzed for radioactivity. One additional mouse was used to collect control samples. The purpose of this study was to assess the distribution of total radioactivity in bone marrow and selected tissues following a single oral administration of [^{14}C]-RH-651 to the mouse.

There were no clinical signs of toxicity. Body weights appeared to be unaffected by dosing. At necropsy, two animals were noted with findings that were suggestive of gavage error.

The highest concentration of radioactivity was found in the blood. Plasma concentrations were substantially lower, indicating that radioactivity partitioned into the red blood cells. Generally, females demonstrated higher levels of radioactivity than males from 3-48 h post-dosing. Mean concentrations of radioactivity declined substantially after 24 h in all tissues except blood. Tissue-to-plasma ratios demonstrated that radioactivity partitioned preferentially from plasma into the tissues (indicated by ratios >1.00), with the exception of bone or bone marrow at 1, 3, or 6 h. Blood-to-plasma ratios increased with time in both sexes, to a maximum at 48 h.

This tissue distribution study is classified **ACCEPTABLE-NON-GUIDELINE**

IV. 2-methyl-isothiazolin-3-one (RH-573)

11. **Metabolism** – Rat; MRID 47154009

EXECUTIVE SUMMARY: In a 96-hour metabolism study (MRID 47154009), ^{14}C-RH-573 (Lot #724.0501, 99.08% radiochemical purity) dissolved in water was administered by gavage to rats at a dose of 5 or 50 mg/kg (4 animals/sex/dose for excretion and metabolite characterization studies; 3 animals/sex/dose for blood and plasma pharmacokinetic studies). A control group was not dosed and was sacrificed at study initiation (1 rat/sex).

In the low-dose group, a mean of 94.02-96.00% of the radioactive dose was recovered by 96 hours post-dosing, with 56.04-65.21% found in the urine, 20.65-29.11% in the feces, 2.49-3.63% in selected tissues, and 4.52-8.37% in cage rinse. In the high-dose group, a mean of 91.71-92.52% of the radioactive dose was recovered, with 47.13-49.59% found in the urine, 33.51-37.39% in the feces, 1.88-2.17% in selected tissues, and 6.12-6.45% in cage rinse. Blood had the highest percent radioactivity in tissues (mean of 1.73-2.49% for the low-dose group, 1.30-1.45% for the high-dose group). Each of the other tissues contained less than 1% of the dose. Most of the administered dose (mean of 80.38-87.29%) was eliminated within 24 hours post-dosing.

Approximately 23 metabolites of RH-573 were detected. The main metabolites in the urine, designated as M1 and M12, contained 20.8 6-23-29% and 9.73-22.67% of the administered dose, respectively. M1 was identified as N-methyl-malonamic acid. M12 was identified as a 3-mercapturic acid conjugate of 3-thiomethyl-N-methyl-propanamide. Urinary metabolites M3 and M9 comprised 4.33-5.63% and 2.42-6.18% of the dose, respectively. The main metabolite in the feces, designated as M2, consisted of four compounds and contained 12.20-25.78% of the administered dose. Each of the other metabolites comprised <3% of the administered dose. Structures were also proposed for two of the components of M2, M3, M9 (composed of two isomers) and seven minor metabolites. A metabolic pathway was proposed for RH-573 in the rat.

In the low-dose group, T_{max} in blood and plasma was reached for both sexes at one hour post-dosing. In the high-dose group, T_{max} was reached at 1.7 hours in males and at 3

hours in females. The elimination half-lives of ^{14}C-label from plasma ($T_{1/2}$ initial) were rapid and ranged from 3 to 6 hours.

This metabolism study in the rat is classified **ACCEPTABLE - NONGUIDELINE** and does not satisfy the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in the rat. The study is upgradable if justification is provided for not collecting expired air samples, or urine samples at 6 and 12 hours post-dosing and/or if these requirements are waived. **The absence of these data does not significantly affect the results of the study.**

12. **Metabolism** – Rat; MRID 471540-10

EXECUTIVE SUMMARY: In a metabolism study of [^{14}C]-RH-573 in the biliary cannulated female rat (MRID 471540-10), ^{14}C-RH-573 (99% pure) was administered by gavage in a single dose of 50 mg/kg. The excretion and metabolite profiles were investigated in bile, urine, and feces. Initially, some 31 metabolites were found and characterized.

The test material was rapidly excreted following a single gavage administration, and was eliminated primarily (about 49%) through the urine. Lesser amounts were excreted (about 29% and 6%) via biliary and fecal routes respectively. Primary excreted compounds in the urine following a single oral exposure included the metabolized parent compound (M-1) as N-methyl malonamic acid and (M-12) as the 3-mercapturic acid conjugate of 3-thiomethyl-N-methyl-propionamide.

The metabolites of RH-573 were a large number of a variety of Phase I metabolites. These consisted of reductive and oxidative cleavage products as well as Phase II products consisting of glutathione and/or glutathione-derived conjugates of Phase I metabolites of RH-573. Glutathione and glutathione conjugates, and di-conjugates with glucuronic acid were also found in the bile samples.

The study was able to provide proposed metabolic pathways for the metabolism of RH-573 in the female rat.

This metabolism study is considered to be **ACCEPTABLE - GUIDELINE** only for metabolite profiling because it satisfies the guideline requirement for the metabolic profiling portion of a metabolism study OPPTS 870.7485.

13. **Tissue Distribution in Mice;** MRID 47154018

EXECUTIVE SUMMARY: In a non-guideline tissue distribution study (MRID 47154018), [^{14}C]-RH-573 (Lot # 395.0113; radiochemical purity 96.7%) in NANOPure® water was administered to 15 CD-1 mice/sex at a dose level of 100 mg base-eq./kg by a single oral gavage at a dose volume of 10 mL/kg. At 1, 3, 6, 24, and 48 h post-dosing, three mice/sex were killed, and blood, plasma, bone, bone marrow, and liver were collected and analyzed for radioactivity. One additional mouse was used to collect

control samples. The purpose of this study was to assess the distribution of total radioactivity in bone marrow and selected tissues following a single oral administration of [^{14}C]-RH-573 to the mouse.

It was stated that several mice were observed to be ill following treatment, and that a number were found dead prior to scheduled termination. One 24 h female was very ill at termination, and was observed to have high concentrations of radioactivity in blood, liver, bone, and bone marrow, and insufficient blood was collected to prepare plasma. Body weights appeared unaffected by dosing. No additional information was provided.

The highest concentration of radioactivity was found in the liver. Generally, males demonstrated higher levels of radioactivity than females. Mean concentrations of radioactivity declined substantially at 24 and 48 h in all tissues. Tissue-to-plasma ratios demonstrated that radioactivity partitioned preferentially from plasma into the tissues and red blood cells (indicated by ratios >1.00), with the exception of bone or bone marrow at 1, 3, or 6 h. Blood-to-plasma ratios increased with time in both sexes, reaching a maximum at 48 h.

This tissue distribution study is classified **ACCEPTABLE – NON-GUIDELINE**

14. ***In Vivo*** **Mammalian Cytogenetics - Erythrocyte Micronucleus assay in Mouse Bone Marrow;** MRID 47154011

EXECUTIVE SUMMARY: In a CD-1 mouse bone marrow micronucleus assay (MRID 47154011), 5 mice/sex/dose were treated once by oral gavage with Kordek®573T (97.5% a.i., lot # B1103) at doses of 0, 10, 50, or 100 mg/kg bw. Two additional mice were dosed in the high dose group per time point. Bone marrow cells were harvested at 24 or 48 hours post-treatment. The vehicle was distilled water. Mitomycin-C dissolved in distilled water was used as a positive control. Preliminary acute oral toxicity study was preformed that utilized six mice/sex/dose treated once by oral gavage at doses of 150, 200, or 250 mg/kg. The LD50 was determined to be 167 mg/kg with 95 percent confidence limits of 137 to 187 mg/kg

Kordek®573T was tested up to the toxicity limit. Two female mice in the 100 mg/kg dose group did not survive the treatment period. Two additional female mice in highest dose group were ataxic, passive, and exhibited labored breathing. No additional signs of toxicity were observed in other female mice or in male mice at 100 mg/kg during the study.

There were no statistically significant increases in the number of micronucleated polychromatic erythrocytes compared to the vehicle controls at any dose level at any time point. Additionally, there was no statistically significant change in the polychromatic/normochromatic ratio at either 24 or 48 hours. The positive control induced the appropriate response.

There was not a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.

This study is classified as **ACCEPTABLE - GUIDELINE** and satisfies the guideline requirement for the Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

15. ***In vitro* Mammalian Cytogenetics**: Cell Chromosome Abberation in Chinese Hamster Ovary (CHO) Cells; MRID 47154019

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics chromosome aberration assay (MRID **47154019**), Chinese hamster ovary (CHO) cells were exposed to Kordek 573T (97.5% a.i., Lot no. B1103) at concentrations of 0.0785, 0.157, 0.313, 0.625, 1.25, 2.50, 5.00, 9.53, 12.7, 16.9, 22.5, 30.0, or 40.0 µg/mL with and/or without metabolic activation for 3 hours in an initial assay; and at concentrations of 0.157, 0.313. 0.635, 1.25, 2.50, 3.75, 5.00, 7.50, 10.0, 12.5, 15.0, or 20.0 µg/mL without metabolic activation for 17.8 hours and 1.25, 2.50, 5.00, 7.50, 10.0, 12.5, 15.0, 17.5, or 20.0 µg/mL with metabolic activation for 3 hours in a confirmatory assay.

Kordek 573T was tested up to limit concentration, 5000 µg/mL, in the first initial assay and to cytotoxic conditions, based on a reduction in overall cell count and/or reduction in mitotic index, in all subsequent assays. Significant increases in chromosomal aberrations were seen in un-activated cultures tested with 9.53 and 12.7 µg/mL, and 3.75 and 7.50 µg/mL in the initial and confirmatory assays, respectively. Significant increases in chromosomal aberrations were seen in activated cultures tested with 12.7 and 16.9 µg/mL, and 7.50 µg/mL in the initial and confirmatory assays, respectively. However, all significant increases in chromosomal aberrations were seen in cultures that could be considered cytotoxic based on reduction in cell count and/or reduction in mitotic index. Positive controls induced the appropriate response. **There was evidence of chromosome aberration induced over background.**

This study is classified as **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement for the Test Guideline *in vitro* mammalian cytogenetics chromosome aberration *OPPTS 870.5375; OECD 473* in Chinese hamster ovary cells.

16. ***In Vitro* Mammalian Cells in Culture Gene Mutation Assay in Cultured Chinese Hamster Ovary Cells**; MRID 47154020

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the hypoxanthine guanine phophoribosyl transferase (HGPRT) locus (MRID 47154020), Chinese hamster ovary cells cultured *in vitro* were exposed to Kordek 573T (97.5% a.i., Lot no. B1103) at concentrations of 0.5, 1.0, 5.0, 10, 15 or 25 µg/mL in the presence and absence of mammalian metabolic activation for 4 hours in a definitive study, and at concentrations of 5.0, 10, 15, 25, or 40 µg/mL in the presence and absence of mammalian metabolic activation for 4 hours in a confirmatory study. S9 metabolic activation was derived from male Sprague-Dawley rat livers induced by phenobarbitol.

Kordek 573T was tested up to cytotoxic limit as determined by the range finding assay. In a preliminary cytotoxicity assay, Kordek 573T was tested up to 5000 µg/mL both in the presence and absence of metabolic activation. Cytotoxicity was noted at ≥50 µg/mL, therefore, doses below 50 µg/mL was used for the mutation assays.

Two mutations assays were conducted with and without metabolic activation. The test substance was not mutagenic in these *in vitro* mammalian cell gene mutation assays in the presence or absence of S9 activation under the conditions of the assay. The positive controls did induce the appropriate response. **There was no evidence of a concentration -related, positive response that induced mutant colonies over background.**

This study is classified as **ACCEPTABLE- GUIDELINE** and satisfies the guideline requirement for the Test Guideline OPPTS 870.5300, OECD 476 for *in vitro* mutagenicity (mammalian forward gene mutation) data.

17. **Unscheduled DNA Synthesis in Primary Rat Hepatocytes / Mammalian Cell Cultures;** MRID 47154025

EXECUTIVE SUMMARY: In an unscheduled DNA synthesis assay (MRID 47154025), primary rat hepatocyte cultures were treated once by oral gavage with 2-methyl-4-isothiazolin-3-one (51.4% a.i., Lot No.: 800IJ123, TD No.: 01-119) at concentrations of 0, 103, 206, or 308 mg a.i./kg. Hepatocytes were isolated from the livers of 4 male rats per group by the two-step *in-situ* perfusion at 2-4 or 12-14 hours post-treatment. Cells were autoradiographed and unscheduled DNA synthesis was evidenced by a net increase in mean net nuclear grain counts, which was observed using an automated colony counter with a microscope. The difference between the cytoplasmic grain count and the mean net nuclear grains, and the percentage of the hepatocytes in S-phase were calculated.

2-methyl-4-isothiazolin-3-one was tested up to cytotoxic concentrations based on the preliminary toxicity test using rat hepatocytes that were treated at dose concentrations of 103, 206, 257, 308, and 411 mg a.i./kg. Toxicity symptoms and mortality data from the preliminary toxicity test were used in determining doses for the UDS assay and behavior similarities between the sexes justifies the use of males only in the UDS assay. In the UDS assay, none of the treatment groups for either timepoint exhibited a positive mean net nuclear grain count and the percent cells with ≥5 net nuclear grain count were below the criteria for a positive response. The positive controls induced the appropriate response. **There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts] was induced.**

This study is classified as **ACCEPTABLE – GUIDELINE** and satisfies the guideline requirement for the Test Guideline OPPTS 870.5550; OECD 482/486 for other genotoxic mutagenicity data.

18. ***In vitro* Bacterial Gene Mutation *Salmonella typhimurium; E. coli/* mammalian activation gene mutation assay**; MRID 471540-26

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 471540-26), strains TA1535, TA1537, TA98, or TA100 of *S. typhimurium* were exposed to 2-methyl-4-isothiazolin-3-one (99.99%, lot # MH32: 72C). Strains TA1535 and TA1537 were tested at concentrations of 0.0001, 0.001, 0.01, 0.1, or 0.25 µg/plate; TA98 was tested at 0.0001, 0.001, 0.01, 0.1, or 1.0 µg/plate; and TA100 was tested at 0.001, 0.001, 0.01, 0.1, 1.0, 2.0, 3.0, 5.0, 10, 25, 50, or 100 µg/plate in the presence and absence of mammalian metabolic activation.

2-Methyl-4-isothiazolin-3-one was tested up to toxic conditions for strain TA100 at concentra-tions of 25 µg/plate and greater under nonactivating conditions. The remaining strains, TA1535, TA1537, and TA98, were tested at limit concentrations of 0.25 and 1.0 µg/plate respectively. The results suggest that there is no mutagenic response induced by 2-methyl-4-isothiazolin-3-one; however, not enough data on test methods were provided to determine if the response were valid. The positive controls did cause a large increase in revertant colonies for the activated test, whereas the non-activated tests were equivalent to the negative controls. **There was no evidence of a concentration related positive response of induced mutant colonies over background.**

This study is classified as **UNACCEPTABLE - NONGUIDLINE** and does not satisfy the guideline requirement for the Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data. The study was completed before the EPA and OECD test guidelines were instituted, however, the intent of the study seems to be in line with the now established criteria. It cannot be determined if information required under the guidelines was either not provided or not collected in this study. The data presented appears to be scientifically valid nevertheless; it is classified as unacceptable because several study details were missing.

IV. N-Methyl Malonamic Acid; Predominate Metabolite of Chloro-2-methyl-3(2H)-isothiazolone and/or 2-methyl-3(2H)-isothiazolone

19. ***In vitro* Bacterial Gene Mutation *Salmonella typhimurium* and *E. coli* mammalian activation gene mutation assay**; MRID 47154013

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 47154013), strains TA98, TA100, TA1535, and TA1537 of *S. typhimurium* and tester strain WP2 *uvr*A of *Escherichia coli* were exposed to n-Methyl Malonamic acid (99.22%/ Lot TOX-NMMA-01 (TD 05-018)), in dimethyl sulfoxide (DMSO) at concentrations of 1.5, 5.0, 15, 50, 150, 500, 1500, or 5000 µg/plate in the presence and absence of mammalian metabolic activation using plate incorporation method.

N-methyl malonamic acid was tested to limit concentration (5000 µg/plate). No inhibition of bacteria growth was noted. The positive controls induced the appropriate

responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **ACCEPTABLE - GUIDELINE** and satisfies the guideline requirement for the Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

V. N-(n-Octyl) Malonamic acid ; Predominate Metabolite of 4,5-Dichloro-2-Octyl-2H-isothiazol-3-one (Kathon 287)

2. ***In vitro* Bacterial Gene Mutation** *Salmonella typhimurium; E. coli/* mammalian activation gene mutation assay; MRID 47154012

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 47154012), strains TA98, TA100, TA1535, and TA1537 of *S. typhimurium* and WP2 *uvrA* were exposed to N-(n-Octyl) malonamic acid in a DMSO solvent (99.18%, TOX-OMA-01) at concentrations of 1.0, 5.0, 15, 50, 150, 500, 1500, or 5000 µg/plate in the presence and in the absence of mammalian metabolic activation using the plate incorporation procedure.

N-(n-Octyl) malonamic acid was tested up to 5000 µg/plate in both the initial toxicity-mutation assay and the confirmatory mutagenicity assay. At all dosages, no bacterial growth was inhibited by test substance toxicity or precipitate in any of the tester strains. The results for both assays confirmed that there were no induced revertants in the presence or absence of S9 mix for any of the tester strains. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of a concentration related positive response of induced mutant colonies over background.**

This study is classified as **ACCEPTABLE - GUIDELINE** and satisfies the guideline requirement for the Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

VI. Chloro-2-methyl-3(2H)-isothiazolone and 2-methyl-3(2H)-isothiazolone (RH - 886); the metabolite N-methylmalonic acid and the hydrolysis product of the metabolite Malonic acid]

20. Three-Month Oral Safety Evaluation in Dogs; MRID 47154027

EXECUTIVE SUMMARY: In a non-guideline subchronic oral safety evaluation study (MRID 47154027), 4 Beagle dogs/sex/dose were exposed to aqueous RH-886 (73.0-75.3% a.i., Batch #s: SW 72/0571 and SW 73/0545) in the diet at dose levels of 0, 150, 500, or 1500 ppm (equivalent to 0/0, 4.66/5.03, 15.25/16.49, and 46.52/49.49 mg/kg/day, [M/F]) for 3 months.

Additionally, 4 dogs/sex/dose were exposed to a combination of RH-35,375 (purity not reported; batch/lot #: MH 24:28A) and RH-00,345 (purity not reported; batch/lot # not

reported) in the diet at doses of 150 + 30 ppm or 500 + 100 ppm, respectively, for 3 months.

No treatment-related effects were observed on mortality, clinical signs of toxicity, body weight, body weight gain, food consumption, neurological/physical examinations, ophthalmoscopy, hematology, clinical chemistry, urinalysis, gross or histopathology parameters at any dose in either sex.

A LOAEL was not observed. The NOAEL is 1500 ppm (equivalent to 46.52/49.49 mg/kg/day, M/F) for RH-886 and 500 + 100 ppm (equivalent to 15.96/16.59 mg/kg/day, M/F) for RH-35,375 and RH-00,345 combined.

This subchronic oral toxicity study is classified as **UNACCEPTABLE-NON-GUIDELINE,** Studies performed by IRDC are not considered to be valid documents by the Agency. Other deficiencies are noted in III B.