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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Silver sodium hydrogen zirconium phosphate (AlphaSan® RC2000): review of Toxicology data.

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TO: Dennis Edwards, Chief / Marshall Swindell, Product Manager
Regulatory Management Branch I / PM Team 33
Antimicrobials Division (7510C)

FROM: Timothy F. McMahon, Ph.D. 3/13/03
Senior Toxicologist
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510C)

THRU: Nader Elkassabany, Ph.D. NE 3/14/03
Acting Team Leader, Team Two
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510C)

and

Norm Cook, Chief 03/14/2003
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510C)

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Action Requested: Review of a subchronic toxicity study in dogs and a 2-generation reproduction study in rats for AlphaSan RC 2000 containing 10% silver sodium hydrogen zirconium phosphate.

Background

Toxicology data for the 10% silver sodium hydrogen zirconium phosphate product were requested by the Risk Assessment and Science Support Branch to support indirect food uses of this product. The registrant (Milliken Chemical Company) has previously submitted acute toxicity, mutagenicity, and subchronic toxicity data for review. To complete the toxicology database for indirect food uses, a subchronic toxicity study in the dog and a 2-generation reproduction toxicity study in the rat were submitted for review. The summaries of these studies are shown below.

Subchronic Toxicity in the Non-Rodent

CITATION: Kuhn, J.O. (2002) Final Report: 90-Day Oral Toxicity Study in Dogs (OPPTS 870.3150) Antimicrobial AlphaSan® RC 2000. STILLMEADOW, Inc. (12852 Park One Drive, Sugar Land, TX). Laboratory Study Number 6664-01, August 30, 2002. MRID 457694-01. Unpublished.

EXECUTIVE SUMMARY

In a subchronic toxicity study (MRID 457694-01), Antimicrobial AlphaSan® RC 2000 (99% a.i.) was administered in gelatin capsules to Beagle dogs (4/sex/dose) at dose levels of 200, 400, or 1000 mg/kg for 90 consecutive days (Groups II-IV, respectively). Due to extreme toxicity, the 1000-mg/kg dose level was reduced to 700 mg/kg on Day 43 in females and on Day 71 in males. Controls (Group I) were not treated.

One Group IV (1000/700 mg/kg) female was sacrificed prematurely (Day 42) due to morbidity. One Group IV male died just prior to necropsy. Clinical signs of toxicity observed in treated animals consisted mainly of diarrhea (sometimes red), soft feces, mucus or gel in feces, and decreased defecation in Group III and IV animals. The study author attributed these findings to excretion of undigested test substance and did not consider them to be toxicologically significant. Although our reviewers agree that these findings may be due to undigested test article, we do consider them toxicologically relevant since Group IV males and females demonstrated reduced body weight and body weight gain. Statistical analysis of body weights on Days 28, 56, and 84 revealed a significant decrease in Group IV female mean body weights compared to Group I females on Day 84. Our reviewers note that all Group IV females had lower body weight than controls at study termination with only one of the dogs gaining weight over the course of the study. Decreased mean body weight and body weight gain also were

observed in Group IV males, but the difference did not reach statistical significance. Group IV males had lower food consumption than Group I males beginning at Week 9, which continued throughout the remaining 4 weeks. Our reviewers note that there was a single Group IV male that ate very little food from Day 62 until the study termination, while the 3 remaining Group IV males ate amounts comparable to the controls. Group IV females consumed less food than the controls beginning at 2 weeks, and food consumption remained lower throughout the remainder of the study duration. Statistical analyses performed at Weeks 4, 8, and 12 revealed that food consumption was statistically significantly decreased at Week 4. By excluding from analysis the females that stopped eating, food consumption was significantly decreased during Week 12 as well. There were several occasions where some Group IV females were force fed, and it appears that the amount of food force fed was included in the food consumption results.

There were no statistically significant differences in hematology at Day 32. There were no statistically significant differences in hematology values in males at Day 60 and at study termination. MID cells (includes less frequently occurring and rare cells correlating to monocytes, eosinophils, basophils, blasts, and other precursor white cells) were statistically significantly higher in all female treatment groups than Group I females at Day 60 and in Group II and IV females at study termination. The study author reported that the results were within normal range and were unrelated to administration of the test article. Our reviewers disagree and note that these MID cells values were outside the reference range provided by the study author (p. 116). In addition, treated females demonstrated slightly elevated granulocytes and reduced lymphocytes at all time points. Only reduced lymphocytes in Group III females at Day 60 reached statistical significance. The study author reported that elevated granulocytes accompanied by a decrease in lymphocytes is often related to stress. Our reviewers believe that this may be the case (Karpinski, et al., 2000; Stefanski and Engler, 1999, abstracts only), but note that this was not observed in the male treatment groups. Our reviewers also note that administering empty gelatin capsules to the controls (Group I) may have aided in the interpretation of this stress-related observation.

The study author reported toxicologically relevant increases in alanine aminotransferase (ALT) and alkaline phosphatase in Group IV males and females over all time points, indicating hepatic injury in high dose animals. The study author stated, and our reviewers concur, that all other changes in clinical chemistry observed were not toxicologically significant or were found only in the dying animals. Group IV males had a statistically significant increase in ALT on Day 32 that fell within normal range, but ALT levels rose over time. Alkaline phosphatase in Group IV males was statistically significant on Days 60 and 90, with a trend of increase over time. The Group IV male that died had extremely high levels of ALT, alkaline phosphatase, GGT, and total bilirubin. When this male was removed from the analysis, there was still an increase in ALT and alkaline phosphatase. Group IV females had high values of CK, ALT, AST, alkaline phosphatase, BUN, and creatine on Day 32. One animal died before the 60 day measurement, but Group IV females still had statistically significant increases in ALT, AST, and alkaline phosphatase. ALT and alkaline phosphatase remained elevated in Group IV females at Day 89.

There were occasional variations in urinalysis parameters that the study author did not relate to treatment. Our reviewers agree that the occurrences were random and unrelated to treatment, but we note that the study did not measure urine volume.

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There were occasional gross pathology findings in Groups I, II, and III that were not considered toxicologically significant. Group IV findings included discolored and/or thick/spongy pancreas and enlarged or discolored lymph nodes. Histopathology investigation pigmentation in the lamina propia of intestines, in liver macrophage, and in the glomerulus of the kidney of Group III and IV animals. These groups also exhibited chronic/granulomatous inflammation in the liver, which was accompanied by hepatic vacuolation and/or necrosis in Group IV animals. The study author attributed the pigmentation to the cosmetic effect of silver and did not consider it to be toxicologically significant; our reviewers agree. Debilitated dogs in Group IV also exhibited renal tubular dilation and necrosis, bronchoinsterstitial pneumonia, cerebral hemorrhage with thrombosis, and thymic atrophy with lymphoid depletion.

Based on the results of this study, the no-observable-adverse-effects-level (NOAEL) is determined to be 400 mg/kg/day, based on chronic granulomatous inflammation of the liver accompanied by vacuolization and necrosis observed at the 1000/700 mg/kg/day dose.

This subchronic toxicity study in dogs is classified as **acceptable**. Although the use of multiple doses at the high-dose level limits the ability to draw definitive conclusions regarding the toxicological effects of the test article at 1000 and/or 700 mg/kg/day in Beagle dogs, it can be conservatively interpreted that the 700 mg/kg/day dose resulted in significant toxic effects from oral administration of the test material. In general, the study was conducted reasonably well, with the exceptions noted in the study deficiencies section of this report, and it provides useful information regarding the effects of the undigested test substance, the cosmetic effects of the test substance, and target organ (i.e., liver) toxicological effects.

Reproductive Toxicity in Rats

CITATION: Wood, E. (2002). Experimental Additive Number 9823-37: Dietary Two Generation Reproduction Study in the Rat. Milliken Chemical, Division of Milliken & Company (Spartanburg, SC). Report No. 656/082, September 13, 2002. MRID 457694-02. Unpublished.

EXECUTIVE SUMMARY: In a two-generation reproduction study (MRID 457694-02), Experimental Additive Number 9823-37 was administered via diet to male and female Crl:CD® IGS BR rats at dose levels of 0, 1000, 5000, or 20,000 ppm. Calculated mean dietary intake pre-mating was reported as 72.5, 362.7, and 1465.4 mg/kg/day for F_0 males, 78.2, 399.9, and 1611.9 mg/kg/day for F_0 females, 100.7, 497.2, and 2017.1 mg/kg/day for F_1 males, and 104.0, 533.7, and 2232.4 mg/kg/day for F_1 females. Animals were exposed to the test article for 75 (F_0 animals) or 76 (F_1 animals) days prior to mating. For both generations, treatment continued through the mating period until termination and included gestation and lactation periods for females. There was clear evidence of parental effects in 20,000-ppm animals, including significantly decreased body weights (F_1 males and females), significantly decreased body weight during gestation and lactation (F_1 females), and significantly decreased mean food consumption (F_1 males and females). Additionally, 20,000-ppm F_1 dams exhibited a significant decrease in the number born and in live litter size on Day 1 *post partum* as well as a nonsignificant decrease in litter size on Day 4 *post partum* and a nonsignificant decrease in the mean number of implantation sites. Other treatment-related observations for both generations included darkened or discolored pancreas (20,000-

and 5000-ppm males and females), darkened mesenteric lymph nodes (20,000- and 5000-ppm males and females), and darkened thymus (20,000-ppm females and 5000-ppm F_0 females only). These pigmentation alterations were reportedly caused from the silver salt found in the test material and were of no toxicological significance. **Therefore, the NOAEL for parental toxicity is 5000 ppm, and the LOAEL for parental toxicity is 20,000 ppm (based on decreased body weights, during maturation, decreased body weights during gestation and lactation, decreased food consumption, decreased number born, and decreased live litter size).** There was clear evidence of offspring effects in 20,000-ppm animals including significant decrease in litter weight (F_1 dose group) and significant decrease in pup weight (F_1 and F_2 dose group). **Therefore, the offspring toxicity NOAEL is 5000 ppm and the offspring toxicity LOAEL is 20,000 ppm (based on decreased pup and litter weights).**

This reproductive toxicity study in the rat is classified **acceptable**. Minor study design and/or reporting deficiencies are not considered to have affected the study findings.