BB-1697

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

007504

SEP 28 1989

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Methylene Bis(thiocyanate). Review of Two Dermal Toxicity Studies in Rats.

EPA I.D. No. CA-61756
Record No. 246075

Project No. 9-1555
Tox. Chem. No. 565

TO: Jim Wilson, PM #31
Registration Division (H7505C)

FROM: John E. Whalan, D.A.B.T., Toxicologist
Section 1, Toxicology Branch I (IRS)
Health Effects Division (H7509C)

John E Whalan
9-11-89

THROUGH: Edwin R. Budd, Section Head
Section 1, Toxicology Branch I (IRS)
Health Effects Division (H7509C)

The Methylene Bis(thiocyanate) Task Force, Buckman Laboratories, Inc. submitted two studies for review entitled:

1. 21-Day Dermal Toxicity Study with Methylene Bis(thiocyanate) in Rats.

2. 13-Week Dermal Toxicity Study with Methylene Bis(thiocyanate) in Rats.

In the 21-day study, there was no systemic toxicity, but there was considerable dermal irritation at doses as low as 10 mg/kg/day. The study was classified Core Guideline, and the NOEL was defined as <10 mg/kg/day.

This study was followed with the 13-week study in which the doses used were as low as 3 mg/kg/day. The latter study was terminated by the Sponsor after less than 3 weeks because of excessive dermal trauma at all dose levels. Although the doses were lower and there were fewer treatments, the dermal irritation was more severe. This discrepancy was not discussed.

Copies of these reviews are attached.

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Reviewed by: John E. Whalan JW 7-11-89
Section I, Tox. Branch I (IRS) (H7509C)
Secondary reviewer: Edwin R. Budd
Section I, Tox. Branch I (IRS) (H7509C)

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DATA EVALUATION REPORT

STUDY TYPE: Subacute (21-Day) Dermal Toxicity Study in Rats

ACCESSION NUMBER: N/A

TOX. CHEM. NO.: 565

TEST MATERIAL: Methylene bis(thiocyanate)
Lot No. 7-0846M (purity not reported)
Amber crystals

MRID NO.: 411119-01

SYNONYMS: Nalco D-1994

STUDY NUMBER(S): HLA 6142-109

SUBMITTED BY: Methylene Bis(thiocyanate) Task Force

TESTING FACILITY: Hazleton Laboratories America, Inc.

TITLE OF REPORT: 21-Day Dermal Toxicity Study with Methylene Bis(thiocyanate) in Rats.

AUTHOR(S): Susan M. Henwood

REPORT ISSUED: January 26, 1989

CONCLUSIONS: No rats died on this study, and there were no abnormal clinical signs observed. Body weight gain, food consumption, clinical pathology values and organ weights were within normal limits. Dose-related signs of dermal irritation included erythema, edema, desquamation, fissuring, subcutaneous hemorrhage, necrotic appearance, atonia, eschar, exfoliation, and pustules/papules. The dermal lesions were more plentiful and more severe in the females.

The high-dose males and all dosed females had crusting of the treated skin which was supported by histopathologic findings of ulceration and chronic inflammation in the high-dose males and all dosed females, and epidermal hyperplasia with hyperkeratosis in the mid and high-dose males and all dosed females.

Although methylene bis(thiocyanate) was not systemically toxic at the doses tested, it was a dermal irritant at all doses. The NOEL is <10 mg/kg/day(LDT).

STUDY CLASSIFICATION: This study is classified CORE GUIDELINE. It was well run and well documented. This study received Quality Assurance review for GLP compliance.

Special Review Criteria (40 CFR 154.7): N/A

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PROTOCOL: Groups of 5 male (227-263 g) and 5 female (162-179 g) acclimated Crl:CD®(SD)BR rats (7-weeks old) were randomly assigned to four groups. They were dermally dosed with methylene bis(thiocyanate) at doses of 0 (vehicle control), 10, 30, and 60 mg/kg/day. The vehicle was a 0.4% aqueous carboxy-

methylcellulose solution (viscosity not specified). Dosing sites were prepared by clipping the fur from a 9 cm square area on the back of each rat. The skin was unabraded. The appropriate test article dose was placed on a gauze pad and moistened with 0.5 ml of the vehicle. The gauze pad was placed on the dosing site, secured with a gauze dressing and nonirritating tape, and occluded with Saran Wrap® and Elastoplast® tape. Vehicle control animals were dosed only with the vehicle. The rats were exposed for 6 hours/day, 4 or 5 days/week, for 3 weeks. The rats received a total of 14 treatments. At the end of each 6-hour treatment period, the dressings were removed and the dosing sites were washed with tepid water and dried with paper towels. The rats were individually housed in stainless steel cages, and food and sterilized, demineralized water were available ad libitum.

The rats were observed twice daily for mortality and moribundity, and once daily for clinical signs. Body weights were measured on the first day of treatment, weekly thereafter, and at the time of necropsy. Food consumption was measured weekly. The dosing sites were graded for dermal irritation prior to applying each day's dose. They were not graded after dose removal. The following clinical pathology evaluations were made on retro-orbital plexus blood collected from the fasted rats on the day of necropsy:

HEMATOLOGY:

Erythrocyte count	MCHC
Hematocrit	Nucleated erythrocytes
Hemoglobin	Total leukocyte count
MCV	Differential leukocyte count
MCH	Platelets

CLINICAL CHEMISTRY:

Blood urea nitrogen	Albumin
Glucose	Albumin/Globulin Ratio
Creatinine	Sodium
Aspartate aminotransferase (AST)	Potassium
Alanine aminotransferase (ALT)	Chloride
Total bilirubin	Calcium
Total Protein	Phosphorus
Globulin	

At the end of the 21-day exposure period, the rats were necropsied and examined grossly. Organ weights were measured for adrenals, brain, kidneys, liver, and testes (with epididymides). Sections of treated and untreated skin, liver, kidneys, and any gross lesions were evaluated histopathologically.

RESULTS: No rats died on this study, and there were no abnormal clinical signs observed other than skin irritation. There were no significant effects on body weight gain or food consumption in either sex.

Dose-related signs of dermal irritation in males were seen at all doses and included erythema, edema, desquamation, fissuring, subcutaneous hemorrhage, and necrosis at all doses; and additionally atonia, eschar, exfoliation, and pustules/papules at the high-dose. The dermal lesions were more plentiful and more severe in the females. The lesions were the same as those found in

the males except that atonia and pustules/papules were found at the low-dose only, and some eschar and exfoliation were found at the mid-dose. No skin trauma was seen in the control animals treated with CMC.

All group clinical pathology values and organ weights were within normal limits. The only dose-related gross lesion was crusting of the treated skin in the high-dose males and all dosed females. Histopathologic lesions were limited to the treated skin and included ulceration and chronic inflammation in the high-dose males and all dosed females, and epidermal hyperplasia with hyperkeratosis in the mid and high-dose males and all dosed females.

Reviewed by: John E. Whalan JW 9-11-89
Section I, Tox. Branch I (IRS) (H7509C)
Secondary reviewer: Edwin R. Budd
Section I, Tox. Branch I (IRS) (H7509C)

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DATA EVALUATION REPORT

STUDY TYPE: (An Aborted) Subchronic (13-Week) Dermal Toxicity Study in Rats

ACCESSION NUMBER: N/A

TOX. CHEM. NO.: 565

TEST MATERIAL: Methylene Bis(thiocyanate)
Lot No. 7-0846M (purity not reported)
Amber crystals

MRID NO.: 411119-02

SYNONYMS: Nalco D-1994

STUDY NUMBER(S): HLA 6142-110

SUBMITTED BY: Methylene Bis(thiocyanate) Task Force

TESTING FACILITY: Hazleton Laboratories America, Inc.

TITLE OF REPORT: 13-Week Dermal Toxicity Study with Methylene Bis(thiocyanate) in Rats. [NOTE: This study was aborted after 3 weeks]

AUTHOR(S): Susan M. Henwood

REPORT ISSUED: March 10, 1989

CONCLUSIONS: This study had to be terminated after less than 3 weeks due to the severity of skin irritation. No rats died on this study, there were no abnormal clinical signs (other than skin irritation), and no effects on body weight gain or food consumption. Dose related signs of dermal irritation in males were seen at all doses and included erythema, edema, atonia, desquamation, fissuring, subcutaneous hemorrhage, necrosis, blanching, eschar, and exfoliation. The lesions were more plentiful and more severe in the females. Gross lesions of the treated skin included red focus areas, abrasion/ulcerations, and crusted areas in a few animals. No other tissues were examined.

STUDY CLASSIFICATION: This study is classified CORE SUPPLEMENTARY. There is a major discrepancy in this study and the preceding 21-Day Dermal Toxicity Study in Rats (HLA 6142-109) performed at the same laboratory. A comparison of skin lesions at the 10 and 30 mg/kg/day doses in both studies clearly shows that the onset of trauma was sooner and more severe in this study, and that the irritation scores were approximately four-fold higher. The study report did not discuss how is it possible that a study using similar procedures, lower doses, and fewer treatments would have to be terminated because of substantially greater skin trauma.

There are two possible explanations. Formulation samples were retained for homogeneity and dose concentration analyses, but the results were not reported (a QA oversight). There may have been a formulation error. Another possibility is that bioavailability may have been greater in this study because the test article was ground and formulated with the aqueous CMC vehicle, whereas in the 21-day study each test article dose (unground) was placed on a gauze pad and moistened with 0.5 ml of the vehicle before it was placed on the dosing site. This study received Quality Assurance review for GLP compliance.

Special Review Criteria (40 CFR 154.7): N/A

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PROTOCOL: Groups of 10 male (267-301 g) and 10 female (173-204 g) acclimated Crl:CD®(SD)BR rats (8-weeks old) were randomly assigned to four groups. They were dermally dosed with methylene bis(thiocyanate) at doses of 0 (vehicle control), 3, 10, and 30 mg/kg/day. The test formulations were prepared by grinding the test article in a mortar and pestel, then mixing measured amounts with a 0.4% aqueous carboxymethylcellulose solution (viscosity not specified). Samples of the test formulations were measured for homogeneity and dose concentration by the sponsor.

Dosing sites were prepared by clipping the fur from a 5 cm square area on the back of each rat. The skin was unabraded. The appropriate dose formulation was applied to the dosing site (2 ml/kg), secured with a gauze dressing and nonirritating tape, and occluded with Saran Wrap® and Elastoplast® tape. Vehicle control animals were dosed only with the vehicle. At the end of each 6-hour treatment period, the dressings were removed and the dosing sites were washed with tepid water and dried with paper towels. The rats were individually housed in stainless steel cages, and food and water were available ad libitum.

The rats were exposed for 6 hours/day, 4 or 5 days/week. The animals were to be dosed for 13 weeks, but the high degree of dermal irritation prompted the sponsor to terminate the 10 and 30 mg/kg groups after study day 15 (11 treatments), and the 3 mg/kg group after study day 19 (13 treatments).

The rats were observed twice daily for mortality and moribundity, and once daily for clinical signs. Body weights were measured on the first day of treatment, weekly thereafter, and at the time of necropsy. Food consumption was measured weekly. These measurements were taken through study day 21 despite the termination of dosing. The dosing sites were graded for dermal irritation prior to applying each day's dose. They were not graded after dose removal.

The study protocol called for clinical pathology, gross pathology, histopathology, and organ weight evaluations, but these were cancelled due to the early study termination. The rats were sacrificed on study day 22, and only the dosing sites were evaluated grossly.

RESULTS: No rats died on this study, and there were no abnormal clinical signs observed other than skin irritation. There were no significant effects on body weight gain or food consumption in either sex.

Dose-related signs of dermal irritation in males were seen at all doses and included erythema, edema, atonia, desquamation, fissuring, subcutaneous hemorrhage, and necrosis at all doses; and additionally blanching, eschar, and exfoliation at the mid and high-dose. The dermal lesions were more plentiful and more severe in the females. The lesions were the same as those found in the males except that blanching was found at the low-dose. No skin trauma was seen in the control animals treated with CMC.

Gross evaluation of the treated skin found red focus areas in a few of the males from all dosed groups. The high-dose females also had some incidence

of red focus areas, abrasion/ulcerations, and crusted areas. Considering that the study had to be terminated on account of the skin lesions, it is surprising that so few gross lesions were found. No other tissues were examined.