10/15/68

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Dr. R. Littmann
10-15-68

B I O M E T

Tri-N-Butyltin Fluoride

Trade Name	:	Biomet
Chemical Name	:	Tri-N-Butyltin Fluoride
Chemical Structure	:	$CH_3-(CH_2)_2-CH_2$ $CH_3-(CH_2)_3-Sn^+ F^-$ $CH_3-(CH_2)_2-CH_2$
Empirical Formula	:	$C_{12}H_{27}SnF$
Company	:	M & T Chemical Inc.
Use	:	Anti-fouling chemical for use in marine paints

Physical and Chemical Properties

Primary Skin Irritation (Tech) : mildly irritating

Primary Rabbit Eye Irritation (Tech) : Ocular irritant, capable of causing blindness if not treated

Primary Rabbit Eye Irritation with Treatment (tech)
: Mild conjunctival irritation in 5/6 rabbits at 24 hours which cleared in all but 1 rabbit by 48 hours.

Primary Rabbit Eye Irritation (Paint Base)
: Mild conjunctival effects in 6/6 rabbits at 24 hours which cleared by 72 hours; therefore not an ocular irritant.

Primary Rabbit Eye Irritation (Paint Base with 20% [illegible]) : Ocular irritant, causes permanent corneal damage if not treated.

Primary Rabbit Eye Irritation with Treatment (Paint Base with 20% Tributyltin Fluoride): : Moderate to severe conjunctival irritation in 6/6 rabbits at 24 hours, which persisted for at least 72 hours.

Vapor Exposure (Paint Base with 20% Tributyltin Fluoride): : No mortality or compound effect at 1) times the maximum concentration.

Subacute Rabbit Dermal (21 days) (Tech): A no effect level not obtained, depressed bone marrow cellularity at 5, 25, and 50 mg/KG. Precancerous skin lesions in 1 and 2 rabbits at site of application of the test material at 25 & 50 mg/KG respectively.

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Dr. A. Fitzhugh
10-15-68

This is a review of the toxicological data on tributyltin fluoride.

Primary Skin Irritation (Tech)

0.5 cc of the test material was applied under a one inch patch to the abraded and intact skin of 6 albino rabbits. The entire trunk of the test animals was wrapped with rubberized cloth and the animals immobilized for the 24 hour exposure period. After 24 hours the patches were removed and the skin reactions evaluated and scored then and at 72 hours according to the method of Draize. A primary irritation index for the chemical was then determined.

Results: At 24 hours one rabbit with intact skin had a 1+ edema and 0 erythema score. At 72 hours the same rabbit had a 1+ erythema and 0 edema score; also at 72 hours one rabbit with abraded skin had a 1+ edema score. The combined score or primary irritation index for this chemical was found to be 0.1, therefore only mildly irritating.

Primary Eye Irritation (Tech)

0.1 cc of the test material was instilled in the conjunctival sac of the eye of 6 albino rabbits. The other untreated eye served as a control. The treated eyes were not washed following instillation. Readings of ocular reactions were made daily for 7 days and scored according to the system of Draize.

Results: Average scores of 80, 80, 80, 83, 91, and 95 for the 6 rabbits were reported for the 7 days of the experiment respectively. In all cases, corneal opacity and conjunctival effects were produced which persisted throughout the 7 day observation period. Therefore the chemical is an ocular irritant and capable of causing blindness if not treated. A second study was performed using, instead of a suspension of the material, 100 mg of powdered Tributyltin Fluoride. The material was instilled directly into the eye of rabbits and the eye held closed for 10 seconds. At 24 hours it was impossible to obtain

readings due to massive discharge and swelling, a condition which persisted through the 72 hour reading period. Therefore, the Draize scores for this test were meaningless. At autopsy after a week, there were congested red conjunctiva, large areas of depilation around the eye, and substantial corneal destruction.

Primary Rabbit Eye Irritation With Treatment (Tech)

100 mg of the powdered test material was instilled into the conjunctival sac of one eye of 6 albino rabbits with forced retention for 10 seconds. The untreated eye served as a control. After 5 minutes the eyelid was held open and gentle lavage with warm tap water continued for 15 minutes using both flooding techniques and irrigation with a syringe. By manual manipulation around the eyelid and with direction of the stream of water the nictitating membrane was caused to function repeatedly during the process. The eye was examined to insure removal of solid particles. The eyes were examined at 24, 48, and 72 hours for evidence of ocular irritation and/or corneal destruction. Ocular reactions were graded and scored according to the method of Draize.

Results: At 24 hours 5 out of 6 rabbits showed 1+ to 2+ conjunctival redness. Two out of 6 rabbits showed 1+ and 2+ conjunctival discharge. Two out of 6 rabbits showed 1+, each, conjunctival chemosis. The 24 hour average score was therefore 4.0. At 48 hours only 1 out of 6 rabbits showed 1+ conjunctival redness. At 72 hours the same rabbit again showed 1 + conjunctival redness. Therefore the 48 hour and 72 hour average score was 0.3. The minimal conjunctival reactions at 24 hours with disappearance of all symptoms except for slight redness in one animal thereafter is at most minimally greater than the reaction to water treatment experience with control animals in the past. There was no evidence of corneal damage, temporary or permanent.

Primary Rabbit Eye Irritation (Paint Base)

The white polyvinyl chloride paint base without [illegible] was tested for primary eye irritation by instilling [illegible] of the test material into the conjunctival sac of one eye of 6 albino rabbits. The untreated eye served as a control. The treated eye was not washed following instillation. The eyes were examined daily for 7 days and ocular reactions were scored according to the method of Draize.

Results: At 24 hours an average score, for the 6 rabbits, of 7 was obtained. At 48 hours an average score of 2 was obtained. There was no evidence of ocular irritation or effect for the rest of the test period. Therefore, since in all cases only mild conjunctival effects were noted, all of which had cleared by the 72 hour observation, the paint base as tested is not an ocular irritant.

Primary Rabbit Eye Irritation (Paint Base with 20% Tributyltin Fluoride)

0.1 cc. of the test material was instilled into the conjunctival sac of one eye of 6 albino rabbits. The untreated eye served as a control. The eye was not irrigated following instillation of the test material. The eyes were examined daily for 7 days and the ocular reactions scored according to the method of Draize.

Results: The average score for the 6 rabbits were 80, 75, 68, 40.5, 53, and 54 for the 7 days respectively. Since in all cases, corneal opacity and conjunctival effects were produced which persisted throughout the 7 day observation period, the compound as tested is an ocular irritant.

Primary Rabbit Eye Irritation with Treatment (Paint Base with 20% Tributyltin Fluoride).

100 mg of the test material was instilled into the eye of 6 albino rabbits with forced retention for 10 seconds. The untreated eye served as a control.

After 5 minutes, the treated [illegible] 15 minutes. [illegible] The eyes [illegible] and scored according to the method of Draize.

Results: At 24 hours, the average score was 15; at 48 and 72 hours the average score was 13.3. Therefore, the ocular irritation produced was found to be greater with the paint formula than with the active ingredient alone.

Vapor Exposure (Paint Base with 2% Tributyltin Fluoride)

A vapor chamber was set up with an unspecified number of rats on a wire screen support in the upper half of the chamber. A panel was adjusted in the vapor chamber and the amount of paint equal to 10 times the maximum calculated for the volume was sprayed on to the panel at extreme range using the recommended dilution of solvent to give a paint spray consistency. Because of the range used it is estimated that the volatile and aerosolized fractions were at 10 times the maximum concentration, and the suspended particulate matter was from 2 to 4 times the maximum condition. The animals were kept in the chamber in a static condition for 1 hour, followed by minimal air admission for 23 hours.

Results: No animals showed any pharmacological response, none died in the subsequent holding period, and at sacrifice there was no abnormal gross pathology.

Subacute Rabbit Dermal (21 days) (Tech)

4 groups of 20 albino rabbits, each group consisting of 5 males and 5 females with abraded skin and 5 males and 5 females with intact skin, had applications of 0, 5, 25, and 50 mg/kg of Tributyltin Fluoride which had been incorporated in petrolatum. The amount of petrolatum utilized as the vehicle for each animal was equal in grams to the animals' body weight in kgs.

The test material in petrolatum was applied to the [illegible] for 5 days each week for 3 weeks. The material was applied over an area of approximately [illegible] sq. cm. of skin surface. The rabbits were then placed in leather restraining jackets in order to prevent their removing the test material from their backs. They were returned to their cages and the exposure was allowed to continue for 24 hours each day. At the end of each exposure period, the jackets were removed from the rabbits and excess test material was removed from their backs using gauze and towels. The animals were then returned to their cages overnight and the test procedure repeated the following day.

The rabbits were under daily observation and were examined for clinical signs indicative of test material effects. Weights were conducted weekly. Autopsies were made on all rabbits which died during the test period. At the conclusion of the investigation all surviving rabbits from each group were sacrificed. Tissues and organs were subjected to gross and histopathologic examination. Absolute organ weights of heart, liver, kidney, spleen, and gonads were obtained. Organ-body weight ratios were not calculated. The left femurs bone was dissected from all rabbits in the control and test groups. Bone marrow was removed and total counts of the cells per bone obtained.

Results:

(A) Body Weight and Food Consumption

A marked mean weight loss was noted in the rabbits at the 50 mg/kg/day dose level over the test period. A slight depression of food intake was also noted in this test group. The rabbits receiving 25 mg/kg/day of the test material were noted to have a slight mean weight loss during the test period. Food consumption at the test level and 5 mg/kg/day compared well with control. The animals at the 5 mg/kg/day dose level gained weight. Inspection of the average weight changes for the 4 groups showed that all test groups and the

control group lost weight [illegible] or the petrolatum base [illegible] was attributed to the fact that [illegible] unaccustomed to their jackets and did not eat [illegible] ditioned to wearing the jackets, [illegible] began to gain weight. However, the group receiving 50 mg/kg continued to show weight losses at all weekly averages.

(B) Mortalities: Two rabbits of the control group died [illegible] of the testing period. Gross autopsies on these animals revealed acute lung inflammation and pneumonitis. One rabbit of the group receiving 5 mg/kg died on the 19th day of the test period. Gross changes at autopsy were parasitic infiltration of the liver and focal pneumonia in the lungs. No deaths occurred in the group receiving 25 mg/kg of the test material. 5 deaths occurred in the group receiving 50 mg/kg of the test material. 4 of these deaths occurred on the 19th test day. The most consistent gross pathology seen in all of these animals was hemorrhage of the lungs and loss of perirenal fat.

(C) Reactions:

No adverse reactions were seen in the control group or the two lower dose levels. The group receiving 50 mg/kg developed considerable erythema and edema of the skin at the site of application about the 7th day of the test period. They appeared to be very sensitive to further applications and by the 14th day a crust had formed over the application area. Some improvement was then noted in the condition of the skin during the final week of application.

(D) Gross pathologic Findings

The only significant gross pathologic changes noted in the test groups were those associated with the skin at the areas of application of the test material. Erythema, edema, and marked thickening of the skin were observed. The incidence

and severity of these observed [illegible] of the test [illegible].

(E) Bone Marrow Counts

Total cell counts per bone in the test group receiving [illegible] material are significantly lower than the control group at the [illegible] level. The mean value for 25 mg/kg dose level is slightly [illegible] mg/kg level, however, both values are significantly different from the control value at the 99% confidence level.

(F) Histopathologic Findings: The only significant histopathologic changes noted were those associated with the skin. The skin changes were characterized by thickening, papillary arrangement, hyperkeratosis, hyperplasia, and necrotic membrane with replacement of epithelium. The skin changes noted in one rabbit at the 25 mg/kg test level and 2 rabbits at the 50 mg/kg test level were characterized by marked hyperplasia, mitotic figures, and presence of some squamous cells(?). These changes of the skin cells showing the characteristics indicative of precancerous lesions were confirmed by a second pathologist after examining additional tissue sections. These precancerous changes were most severe in tissues originating from rabbits receiving applications of 25 50 mg/kg.

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The toxicological [illegible] has been reviewed. The only studies available [illegible] irritation study, several eye irritation studies [illegible] and a subacute dermal study. The compound is a skin irritant and a severe eye irritant, capable of causing permanent corneal destruction and scarring resulting in blindness if not treated. In rabbits with treatment for 15 minutes after a contact interval of 5 minutes, the compound was found to cause only conjunctival irritation.

When tested subacutely dermally for 21 days the compound was found to cause weight loss and skin irritation at the site of application at the two highest dose levels tested. At all three dose levels tested the compound was found to depress bone marrow cellularity and to cause histopathologic alterations of the skin consisting of thickening, papillarity, hyperkeratosis and hyperplasia. Early cell changes indicative of precancerous lesions at the site of application were noted by two different pathologists in two rabbits at the [illegible] KG dose level and one rabbit at the 25 mg/KG dose level. Therefore a no effect level was not obtained for this compound dermally.

In view of these findings registration of this compound should be withheld until additional data is made available which disproves the apparent carcinogenicity of the compound. These studies should be in the form of a repeated subacute dermal study, a chronic dermal study, studies using rats or mice in addition to a further study on rabbits, or studies testing the compound dermally at lower dose levels. Acute studies such as an oral LD_{50}, dermal LD_{50}, inhalation, and a subacute oral feeding study, along with a complete physical and chemical data sheet, should also be made available.