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

005165

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MAY 30 1986

MAY 30 [redacted]

MEMORANDUM

SUBJECT: Hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine
Bioban GK; Glokill 77
EPA File Symbol 48301-RU

Caswell No. 481C
Project No. 902

FROM: Joycelyn E.Stewart, Ph.D. 5/27/86
Review Section No.VI
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: John Lee, PM 31
Disinfectants Branch
Registration Division (TS-767C)

THRU: Jane E. Harris, Ph.D. 5/27/86
Section Head No. VI
Toxicology Branch
Hazard Evaluation Division (TS-769C) 5/30/86

Registrant: Angus Chemical Company
Northbrook, IL 60062

Action Requested:

1. Review toxicology data for registration of chemical as antimicrobial agent in metalworking fluids and paints.

2. Review label

3. Indicate additional studies required for registration

Also included in the submission is a request for waiver of data for teratology, mutagenicity, and oncogenicity studies.

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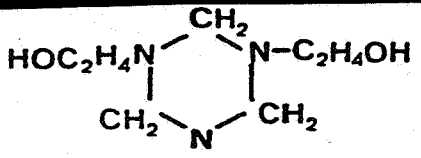
MANUFACTURING PROCESS INFORMATION NOT INCLUDED

Recommendations:

This is the first application for registration of Bioban GK, hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine. The proposed use is for controlling microbial growth in water-based metalworking fluids and in latex emulsions and water dispersible paints. The product is to be added at concentrations of 0.04 to 0.2 percent (400 ppm to 2000ppm) to use-diluted metalworking fluids; and at concentrations of 0.1 percent (1000 ppm) to latex emulsions and water dispersible paints at any convenient time during the manufacturing process. It is supplied as a 78.5 percent solution of the active ingredient in water. The technical grade of the active ingredient (TGAI) is the end use product (EUP). The compound is a formaldehyde releaser. Its effectiveness as an antimicrobial agent is a result of its ability to slowly release forformaldehyde. It is not volatile at normal ambient temperatures.

[redacted]



The acute toxicity studies (acute dermal, eye irritation, primary dermal irritation, dermal sensitization) submitted are adequate for labelling purposes. The compound is a Category I eye irritant and the proposed "Danger" label is adequate. The compound appears not to be a dermal irritant and is not a dermal sensitizer. No acute inhalation study was submitted. The acute oral LD_{50} study was classified as Supplementary.

The registrant has requested that data from teratology studies be waived based on (1) the low potential for exposure to the chemical because it would only be handled by personnel in industrial workplaces who maintain metalworking fluid systems and (2) the chemical is a formaldehyde releaser. Formaldehyde was found not to be a teratogen by the FDA consensus workshop on formaldehyde.

The teratology data for hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine cannot be waived based on th claimed low potential for exposure, because the claim does not preclude acute exposure of females to the compound. Subdivision F of the FIFRA Guidelines states in §83-3 that teratology studies are required when use of a pesticide under commonly recognized practice may reasonably be expected to result in significant exposure of acute duration

to human females. In addition, it might not be reasonable to extrapolate data from formaldehyde to hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine because the absorption and metabolism may not be the same and thereby the teratogenic potential may differ from that of formaldehyde.

The mutagenicity data submitted are not adequate to determine the mutagenic potential of the test compound. The request for waiver of mutagenicity data to support the registration of the chemical is based on the registrant's expectation that the response of hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine to mutagenicity testing would "mirror" that of formaldehyde. If that rationale is accepted, the compound would have to be considered a mutagen, because formaldehyde's mutagenic potential has been shown in a number of tests (Federal Register 29:(101), 21874, May 23, 1984).

Notwithstanding NCI's rejection of the compound for oncogenicity bioassay, Subdivision F, §83.2 of the FIFRA Guidelines states that data from oncogenicity testing are required when considerable exposure to the pesticide is to be expected over some portion of a lifespan. Moreover, formaldehyde has been demonstrated to induce tumor formation by the inhalation route. It is not clear what degree of exposure to formaldehyde by the inhalation route would result from the uses of Bioban GK in metalworking fluids and latex paints.

Based on these considerations, the request for waivers of data for teratogenicity, mutagenicity, and oncogenicity cannot be granted. However, the oncogenicity studies may be deferred pending the submission of teratology, mutagenicity and exposure information.

The studies submitted do not satisfy all the data requirements for registration of hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine as an antimicrobial in metalworking fluids and latex paints. The toxicological potential of the compound should be evaluated initially in a 21 day dermal toxicity, a gavage teratology study in one species (rat or rabbit), and a battery of mutagenicity studies (to determine gene mutations, chromosomal aberrations and other genotoxic effects, e.g. DNA damage/repair). If the results of these studies raise significant concerns,the Agency reserves the right to require a second and perhaps a third tier of toxicity testing. The second tier of studies would consist of: subchronic oral rodent and nonrodent studies, a second teratology study, and a dermal absorption study. The third tier would be chronic feeding studies in two species(rodent and nonrodent), and rodent oncogenicity and reproduction studies.

The requirement for the 21 day dermal toxicity study has been satisfied.

The following data gaps are identified at the present time for hexahydro-1,3,5-tris (2-hydroxyethyl)-s-triazine: acute oral LD_{50} study, acute inhalation LD_{50} study, gavage teratology study in one species, and a battery of mutagenicity studies to test gene mutations, chromosomal aberrations and other genotoxic effects, e.g. DNA damage/repair.

cc. EAB/ David Severn, Ph.D

Studies Supporting this Action

Study	Toxicity Category	Classification
Acute Oral Toxicity in Mice		Supplementary
Summary of Safety Data on Groton		Supplementary
Acute Dermal Toxicity Screen in Rabbits of Bioban GK	III	Minimum
Ocular Irritation Study in Rabbits	I	Minimum
Primary Dermal Irritation Study in Rabbits	IV	Minimum
Draize Skin Test Dermal Sensitization	Not a dermal sensitizer	Minimum
Sensitizing Effect of Groton BK in the Guinea Pig		Supplementary
21-Day Dermal Toxicity Study in the Rat	NOEL for systemic toxicity = 1000 mg/kg LEL for dermal toxicity < 100mg/kg	Minimum
Guinea Pig Maximization Test		Supplementary
Study of Biocides for the Selection of Candidates for Carcinogen Bioassay		Supplementary
Ames Test	Positive in TA 98 with m.a.; Positive in TA 1538 with and without m.a.	Unacceptable
Micronucleus Test in Rats	Negative	Unacceptable

Glokill 77: Acute Oral Toxicity. Consultox Laboratories, London, England, March 1973. Acc. Number 260195

Materials and Methods

Glokill 77 (purity unstated) was diluted to 20 percent in distilled H_2O and administered orally by intubation to fasted Tyler's Original strain white mice weighing 20 $\pm$ 2 grams. After administration of the test compound, food and water were offered ad libitum. A preliminary range-finding study was carried out using two mice/dose level. Doses used were 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mL/kg of the test compound. Animals were observed for 1 week postdosing.

Based on the results of the preliminary study (all mice administered 2.0 mL/kg and above of the test compound died), a final assay was performed on 5 groups of mice using 10 mice (5 males and 5 females) per dose level. Doses used in this study were 0.75, 1.00, 1.50, 1.75, and 2.00 mL/kg of the test chemical. Animals were housed according to dosage levels and were observed for mortality and/or toxic signs for 14 days.

Results

In the preliminary study no deaths were reported among the mice receiving 0.5 mL or 1 mL/kg. In the main study, the mortality was 0:10; 2:10; 7:10; 8:10; and 10:10 for the 0.75, 1.00, 1.50, 1.75, and 2 mL/kg of the test chemical. Initial signs of toxicity were reported to occur within 30 minutes of administration of the test compound, in the form of disoriented

locomotion, loss of activity, and coma. All deaths were reported to occur within 24 hours. The oral LD_{50} was calculated to be 1.30 (1.14 to 1.48) mL/kg.

Discussion and Conclusions

The study, as reported, suffers from several deficiencies. No animal weights were recorded, daily observations for toxic signs were not made, surviving animals were not examined and necropsied, the LD_{50} for each sex was not determined, and the method used to calculate the LD_{50} was not reported. The LD_{50} was reported in mL/kg, and the equivalent mg/kg dose was not calculated. The study cannot be given a Toxicity Category.

Core classification: Supplementary.

Summary of Safety Data on Grotan - Broad Spectrum Bactericide. Sterling Drug Co., Montvale, NJ. Acc. No. 260195

A summary of toxicology studies on Grotan done between 1963 and 1968 is included in the submission, as follows:

ACUTE ORAL TOXICITY IN RATS

LEBERCO 44432 (April 19, 1966)

"The Oral LD_{50} (in rats) was calculated to be 0.8 ml. per Kg of fasted rat with a 19/20 confidence limit of 0.678 ml. to 0.944 ml. per Kg of fasted rat." (This is a volume dose).

LEHN & FINK (January 15, 1963)

The Oral LD_{50} for rats was found to be 0.58 gm/Kg. (This is a weight dose.)

This toxicity was determined by the method of Litchfield and Wilcoxon, J. Pharmacology and Experimental Therapeutics 96: 99, 1949.

ACUTE DERMAL TOXICITY ON RABBITS

LEBERCO 65516 (June 22, 1966)

No animals died following doses of 1.9 ml., 3.0 ml. and 4.76 ml. per Kilogram of body weight, being applied and allowed to remain in contact for a 24-hour period. There was evidence of burns and concurrent changes in the structure of the skin as might be expected. After 14 days, there were no pathological changes in any internal organs.

This study was done in accordance with the provisions for skin testing, as stated in the Federal Hazardous Substances Labeling Act, Paragraph 191.10.

SUB-ACUTE DERMAL TOXICITY ON RABBITS

LEBERCO 66898 (September 9, 1966)

"Fifteen applications of GROTAN diluted to 0.15%, 0.75% and 1.5% in 3% Trim Cutting Oil, to the clipped intact and abraded backs of rabbits (5 days a week for 15 applications) did not produce any significant pathological changes in any of the test animals."

This was done by the method described by Draize, et al. in Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, published by the Association of Food and Drug Officials of the U.S.A., in 1959.

EYE IRRITATION

Food and Drug Research Laboratories. Report 88759 February 16, 1968.

Concentrated GROTAN was found to be irritating to the eyes of rabbits. However, if immediately after contact the eye was thoroughly flushed with water, no irritation develops.

INHALATION STUDY ON GUINEA PIGS

LEBERCO 76924 (July 11, 1967)

"As concluded by our pathologist "no treatment related lesions" were present in the test animals following exposure to

(GROTAN 0.15%, Cutting Oil 3% in water or GROTAN 1.5%, Cutting Oil 3% in water), 8 hours a day, 5 days a week for 4 weeks."

This was done by the technique of Lowsma, Jones and Prendergast described in Toxicology and Applied Pharmacology 9: 71, 1966. The protocol and results were approved by the U.S.D.A. and the U.S. Public Health Service.

HUMAN REPEATED INSULT PATCH TEST

Food and Research Laboratories (28 April 1968)

"Under completely non-occluded conditions, Lehn & Fink Products Co. - Grotan - Broad Spectrum Bactericide was not capable of eliciting visible skin changes consistent with the criteria deemed characteristic of a primary irritant, fatiguing agent or sensitizer in any of the 101 subjects."

"In the opinion of the investigator, Grotan - Broad Spectrum Bactericide may be considered safe to use in contact with the skin insofar as primary irritation, fatiguing or sensitization are concerned if the conditions of contact do not exceed those of the test procedure."

"Insofar as sensitization is concerned, the extrapolation of these results to a general population is limited statistically by the number of test subjects. In this case since 101 subjects were used, we may predict with 95% certainty that at least

96.38% of a general population will not be sensitized by this material."

Conclusions

These studies could not be evaluated because no data were included.

The Core Classification is Supplementary.

Acute Dermal Toxicity Screen in Rabbits of Bioban GK for Argus Chemical Co. Hill Top, Cincinnati, Ohio, Research Project No. 85-0866-21, August 30, 1985. Acc. No. 260195

Materials and Methods:

Bioban GK described as a yellow liquid was applied to the prepared skin of 10 New Zealand White rabbits (5 male and 5 female) at a dosage of 2 g/kg. Animals weighed 2071 to 2667 grams. The test material was applied undiluted in a sleeve of rubber dental dam which was wrapped with a layer of gauze and tape. Animals were secured for 24 hours in a Newmann harness. At the end of the exposure period, the test material was removed by sponging the area with a moist towel.

Animals were observed for toxicity at 2 1/2 hours post-compound application, and twice daily thereafter for 14 days. Observations for application site irritation were made daily. Body weights were taken at day 7 and at study termination. At study termination, gross necropsy was performed on all animals.

Results Reported:

All animals survived the study. Diarrhea, emaciation, fecal stains, and decreased defecation were reported in one animal. This rabbit lost 830 grams during the study. All other animals exhibited normal appearance and behavior and gained weight during the observation period. Erythema, edema, desquamation, necrosis, and coriaceousness were reported.

Gross necropsies performed at termination of the study showed two animals with pale adrenals, and one with mottled lungs, distended gall bladder with green fluid, body fat depletion, and green spleen.

Discussion and Conclusions

Based on the results reported, the acute dermal LD_{50} of Bioban GK is > 2 g/kg. A deficiency in the study is that no individual skin irritation data were supplied.

Toxicity Category is III.

Core Classification: Minimum.

Determination of the Degree of Ocular Irritation Caused by Glokill 77 in the Rabbit. Study No. 371/8408. Safe Pharm Laboratories Ltd., Derby, England, September 3, 1984.

Materials and Methods

Six albino New Zealand White rabbits 12 to 16 weeks of age weighing 2.53 to 3.10 kg were administered 0.1 mL of Glokill 77 undiluted, into the right eye by gently pulling the lower lid away from the eyeball to form a cup. Upper and lower lids were held together for 1 second after administration of the test compound. The left untreated eye served as control. Animals were observed for eye irritation by the Draize system at 1, 24, 48, and 72 hours following treatment. Eyes were examined with a standard ophthalmoscope.

Interpretation of Results

An animal was considered to have exhibited a positive response if the test material produced, at the 24-, 48-, or 72-hour readings, ulceration or opacity of the cornea, inflammation of the iris, or produced in the conjunctivae an obvious swelling with partial eversion of the lids or diffuse crimson-redness with individual vessels not easily discernible.

If two or more animals exhibited a positive reaction the test material was classified as a positive eye irritant. If none or one animal only exhibited a positive reaction the test material was classified as a negative eye irritant. If

irreversible damage to the eye was demonstrated in one rabbit the test material is defined as a corrosive to the eye. In addition, the test material was assigned a Group Total Score for eye irritation.

Results Reported

Corneal opacity was reported in all animals at the 24-hour posttreatment reading. This increased in severity at up to 72 hours. Iritis, conjunctival redness and discharge, chemosis, necrosis, hemorrhage of conjunctiva and nictitating membrane of increasing severity were observed in all treated eyes. Due to the severity of the reactions all animals were sacrificed after the 72-hour reading. The total Draize Group Score was calculated to be 239. The test compound was classified as a positive eye irritant.

Discussion and Conclusions

The results reported support the investigators' conclusions that Glokill 77 is a positive irritant/corrosive to rabbits eyes.

Toxicity Category is I.

Core Classification: Minimum.

Primary Dermal Irritation Test: Determination of the Degree of Primary Cutaneous Irritation Caused by Glokill 77 in the Rabbit. Safe Pharm Laboratories, Ltd., Derby, England, Study No. 317/8505.

Materials and Methods

Six albino New Zealand White rabbits 12 to 16 weeks of age, 2.32 to 2.90 kg were administered Glokill 77 by dermal application on prepared sites on the back of each animal. Animals were acclimatized to the laboratory for 5 days, and had healthy intact epidermises before being placed in the study. A quantity of 0.5 mL of the test material was applied to the shaved skin on a 2.5 cm gauze square and was held in place by adhesive strapping over which an elastic corset was placed. The exposure period was for 4 hours after which the test material was removed by swabbing the area with distilled H_2O. Animals were examined for toxic signs and for irritation 1 hour following removal of the patches and at 24, 48, and 72 hours thereafter. Irritation was scored according to the Draize procedure.

Results

Slight erythema and edema formation (Draize Score = 1) was reported in three out of six rabbits. Slight erythema only (Draize Score = 1) was reported in one additional rabbit. These effects were observed at the 1-hour reading only. No other signs of toxicity were reported.

Discussion and Conclusion

Based on the results presented, the test compound is not a primary skin irritant.

The Toxicity Category is IV

The Core Classification is Minimum.

Glokill 77: Draize Skin Test, Guinea Pig Sensitization Test. Consultox Laboratories Ltd., London, England, CL74:74:1024, July 1974. Acc. No. 260195

Materials and Methods

a. Draize Skin Test

The test material, Glokill 77 (Batch No. K 249) was applied to the prepared skin of two groups of four New Zealand White rabbits. The test compound was diluted with distilled H_2O to concentrations of 0.1 percent (representing the normal usage concentration) and 1 percent. A sample of 0.5 mL of each concentration of the test compound was applied to both intact and abraded skin under 1-inch gauze pads secured with adhesive tape and covered with a "Stockinette"sleeve covering the entire back. Patches were removed after 24 hours,and reactions evaluated according to the Draize scale. A second evaluation was done at 72 hours post compound application and both scores averaged to give a Primary Irritation Index.

b. Guinea Pig Sensitization Test

The dermal sensitization potential of Glokill 77 was determined using a modification of the Draize technique (Draize, Woodward, and Calvary 1944 NAS-NRC 1964). The test material was diluted with distilled water to a concentration of 0.1 percent, and 0.1 mL of the solution was injected intradermally into the shaved back of six male Hartley guinea pigs weighing 300 to 400 g, every other day for nine injections, using a different injection

site each time. Injection sites were examined for erythema and edema 24 hours after each injection using the Draize scale. Two weeks after completion of the sensitizing injections, animals were given a single intradermal challenge dose of the test compound. Twenty-four hours later the test sites were examined for erythema and edema. The effects produced by the primary doses were compared to those produced by the challenge dose to determine whether sensitization had occurred.

Results

In the Draize Skin Test, one animal given the 0.1% solution of Glokill 77 showed slight erythema (Draize Score = 1) on the abraded skin at 24 hours. No erythema or edema was reported in the intact skin. Primary irritation score was 0.06. In the animals administered the 1 percent solution of Glokill 77, slight erythema (Draize score = 1) was reported in the intact skin of one rabbit at 24 hours postadministration of the test compound and in four abraded skin sites at 24 hours postadministration of the test compound. None of the reactions persisted beyond 24 hours. The primary irritation index was calculated to be 0.44.

In the Guinea Pig Sensitization Test, slight to well-defined erythema was observed (Draize score = 1 to 2) in all test animals. Very slight or no edema (Draize score = 0 to 1) was reported in the test animals. Administration of the challenge dose of the test compound did not produce any additional erythema or edema (Draize score 1 to 2 for erythema; 0 to 1 for

edema). The investigators concluded that Glokill 77 was not a sensitizing agent.

Discussion and Conclusions

The data presented support the conclusion that Glokill 77 is not a sensitizing agent. The deficiencies noted in the study were that no individual observations for toxic signs were made and no body weights were taken. Also, the challenge dose used was not stated. It is assumed to be similar to the sensitizing dose.

Toxicity Category

1. Dermal Irritation	IV
2. Dermal Sensitization	Not a dermal sensitizer
Core Classification	Minimum

Sensitizing Effect of Groton BK in the Guinea Pig. P. Poitou and B. Marignac. Contact Dermatitis 4:166, 1978.

This literature reference is a short communication on the sensitizing effect of hexahydro-1,3,5-tris(2 hydroxyethyl)triazine (HTHT, Grotan BK) used as a preservative in coolant oils.

This sensitizing potential of the compound was determined using Magnusson and Kligman's technique on 20 female Hartley albino guinea pigs weighing approximately 400 grams.

Sensitization was produced by intradermal injections of the test compound (0.5% in olive oil) and of complete Freund's adjuvant followed 1 week later by cutaneous application of challenge of 20 percent of test compound in petrolatum.

The effect was tested 2 weeks later by topical application of 0.1, 1.0 2.0 and 4 percent of the compound in vaseline and the sensitizing potential was low. However, when the compound was injected intradermally, or applied after treatment with sodium lauryl sulphate or dermal scarification, the sensitizing potential of the compound increased to 100 percent.

Discussion

Based on the information presented in this paper, Grotan BK may have a sensitizing effect. The degree of dermal sensitization seemed to depend upon the condition of the skin surface. This paper did not give any details of the procedures used, how the skin reactions were graded, or any individual animal data.

The study is classified as Supplementary.

Glokill 77, 21 Day Dermal Toxicity Study in the Rat. Safe Pharm Laboratories, Ltd., Derby, England, Study No. 508/8411, July 19, 1985. Acc. No. 260195

Materials and Methods

Forty Sprague-Dawley rats, 7 weeks of age, were randomly assigned to four treatment groups, each consisting of five males and five females. The test material, Glokill 77, (Batch TS-704, 94.8% ai) a clear viscous liquid was applied to the prepared skin (10% of the body surface area) of the dorsolumbar region at dosage levels of 100, 500, and 1000 mg/kg for 21 days. The test material was used undiluted as supplied. One group served as controls and was treated with sterile distilled water in similar fashion as the test compound-treated animals. Identity and stability of the test material were not determined by the investigating laboratory. The test compound was held in contact with the skin by a porous gauze dressing covered with an Elastoplast elastic adhesive bandage. At the end of each 6-hour exposure period the bandage and dressing were removed and the exposure site washed and dried.

The test site of each animal was observed daily for local dermal irritation. Animals were observed daily for toxic signs. Animals dying during the study were subjected to a detailed postmortem examination, and tissues were preserved for histopathological examination.

Body weight was recorded immediately prior to the start of the study, and twice weekly thereafter. Food consumption was

recorded weekly. Water consumption was monitored daily.

Hematology and serum biochemical determinations were conducted on a separate group of 10 animals (5 male, 5 female) from the same stock prior to start of the study, and on all test and control animals at termination of the study. Determinations were done for hemoglobin, erythrocytes, hematocrit, mean corpuscular volume, leukocytes (total and differential) and prothrombin time. Mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were calculated. Blood chemistry values were determined for blood urea nitrogen, total protein, albumin, A/G ratio, creatinine, total bilirubin, alkaline phosphatase, SGPT, SGOT, gamma glutamyltranspeptidase, glucose, calcium, potassium, sodium chloride, and phosphate.

At termination of the study, all surviving animals were necropsied, and terminal weights taken on liver, kidney, adrenals, and gonads. Histopathology was done on liver, kidney, lymph nodes, skin (treated and untreated) and organs showing lesions or change in size. Skin was examined histologically in all treatment groups, since compound-related changes were observed in the high-dose group.

Results Reported

All animals survived the study. No toxic signs were reported except for local dermal irritation, which was dose related and increased in severity with the duration of the study. All animals gained weight during the study; however,all groups

of treated males gained less weight than corresponding controls. Male weight gain in all groups was approximately 70% of the control male weight gain. The weights observed for all three treatment groups was within the range normally observed in the investigators' laboratory, and therefore were not considered treatment related. Weight gain was comparable in control and treated females.

No difference in food consumption was reported between control and treated males, but during week 1, the mid and high-dose female food consumption was significantly decreased ($p < 0.05$) when compared to the controls. No intergroup difference in water consumption was reported.

Mean corpuscular volume was reduced in the low- and high-dose males, and hemoglobin was reduced in the low- and high-dose females, but the reductions were not considered biologically significant since they were within the normal range of values for Sprague-Dawley rats in the investigators' laboratory.

The following statistically significant changes were noted in the treated animals when compared to the controls ($p < 0.05$): increased protein in mid and high dose males and high dose females,increased sodium in all treated males,increased phosphate in mid and high dose females, increased AST in mid and high dose males,decreased A/G ratio in mid and high dose males and high dose females, and decreased chloride in mid and high dose females. These changes were considered biologically insignificant because the values observed were within the range observed in the

in the animals used in the hematology and biochemical screens.

Except for multifocal scab formation at treatment sites at all dose levels, no macroscopic abnormalities were reported. Absolute adrenal weight was significantly decreased in low and and high-dose males and was reported to be due to two control animals with high adrenal weights. No difference was reported in relative organ weights. Treatment-related histopathological changes were reported at treated skin sites, but in no other tissues examined. Histopathological findings were: dose related epidermal ulceration, acanthosis and dermal inflammatory infiltration with occasional necrosis and hemorrhage in the treated animals.

Discussion and Conclusions

Dermal administration of Glokill 77 at dose levels of 100, 500 and 1000 mg/kg to the intact skin of male and female Sprague-Dawley rats for 21 days, produced no mortality, no adverse effects on food or water consumption, or on hematological or serum biochemical parameters. Although body weight gain was reduced in the treated males when compared to the controls, the reduction was equal in all treated groups (approximately 70% of control), and no dose response was apparent. Food consumption was reduced in all male groups over the course of the study, but more so in the treated animals. Terminal food consumption was reduced to 92.5, 85, 87, and 85.2 percent of the first week's food consumption the control, low, mid and high dose groups respectively,

and so the decrease in weight gain in the treated males was probably related to the slight decrease in food intake among the treated males, rather than to an effect of the test compound.

Based on the dose related epidermal ulceration and dermal inflammation reported at the treated skin sites, a NOEL for local dermal toxicity was not obtained in this study. The NOEL for systemic toxicity is 1000 mg/kg, the HDT.

Core Classification: Minimum

Guinea Pig Maximization Tests with Formaldehyde Releasers-Results from Two Laboratories. K. E. Anderson, A. Boman, K. Hamann, and J. Wahlberg. Contact Dermatitis 10:257-66, 1984.

Materials and Methods

The Guinea Pig Maximization Test was used to evaluate the sensitizing potential of formaldehyde and six formaldehyde releaser in laboratories in Copenhagen and Stockholm. Female albino guinea pigs of SSC:Al strain were used in Copenhagen, while female Dunkin-Hartley guinea pigs were used in Stockholm. The procedure used was that of Magnusson and Kligman (1970). Pre-testing for irritancy was done on separate animals to establish a moderate irritant dose concentration for induction and a non-irritant concentration for challenge. The tests were performed in series, up to 5 groups of 20 guinea pigs. In each series 20 animals were "sham" treated to obtain blind readings of challenge reactions and similar stress on both control and test animals. For the topical induction patch test, filter paper was used, impregnated with the test material, and mounted on Leukoflex or Blendrum.

The test compounds were applied in three concentrations: 1 percent, 0.5 percent, and 0.1 percent. The patch tests were sealed by Acrylastic. Challenge reactions were read blind at 48 hours. The grading scale used was: 0 = no visible change; 1 = discrete or patchy erythema; 2 = moderate and confluent erythema; 3 = intense erythema and swelling. The formaldehyde-releasing chemicals used were:

Forcide 78: 1,3,5-tris(ethylhexahydro-triazine) and 1,3,5-tris(hydroxyethyl)hexahydrotriazine(1:1).

Gerwall 115: N,N'-methylene bis N'-1(hydroxymethyl)2,5 dioxo-4-imidazolidinyl urea.

Grotan BK: 80% 1,3,5-tris(hydroxyethyl)hexahydrotriazine

Grotan OX: N,N-methylene-bis-5-methyl-oxazolidine

KM 200: 1,3,5-tris(hydroxyethyl)hexahydrotriazine

Preventol D2: Mixture of hydroxymethylene and polyhydroxy-methylene monobenzylether.

Results were analyzed by Fisher's Exact Test.

Results

The sensitization frequency of formaldehyde and Grotan BK on challenge day 21 was different in the two laboratories, as shown in the following table.

	Copenhagen	Stockholm
formaldehyde	50%(27%-73%)	95%(74%-100%)
Grotan BK	20%(6%-44%)	74%(49%-91%)

Numbers in parentheses indicate the 95% confidence limits.

In formaldehyde-sensitized animals challenged with Forcide 78, Preventol D2, and Grotan BK, in Copenhagen, the frequency of positive reactions were reported to be:

Test Compound	Challenge Day	Animals with Positive Response
Formaldehyde 1% aq.	21	10:20
Forcide 78 1% pet.	35	5:20
Preventol D2 1% pet.	35	8:20
Grotan BK 1% pet.	35	3:20
Formaldehyde 1% aq.	42	12:20

The frequency of positive reactions to formaldehyde in guinea pigs sensitized to Forcide 78, Grotan BK, and Preventol D2 is shown in the following table from the investigators' report.

Test Compound	Challenge Day	No. of Animals Giving Positive Response
Forcide 78 animals:		
Forcide 78 1% pet.	21	5:20
formaldehyde 2% aq.	28	13:15
Grotan BK animals:		
Grotan BK 1% pet.	21	4:20
formaldehyde 1% aq.	35	2:20
formaldehyde 1% aq.	42	6:20
Grotan BK 1% pet.	42	4:20
Preventrol D2 animals:		
Preventol D2 1% pet.	21	4:19
formaldehyde 2% aq.	35	10:19
formaldehyde 2% aq.	42	3:19

Generally, more animals were sensitized to aqueous solutions of the test compound. Analysis of the formaldehyde releasers demonstrated a higher percentage of formaldehyde release from aqueous solutions of the test compounds.

Discussion and Conclusions

The data contained in this submission demonstrate that the sensitizing potential of formaldehyde and six formaldehyde releasers varied according to the testing laboratory, the animal strain, the concentration of the test chemical, and the vehicle used.

Formaldehyde sensitized 50 percent of the guinea pigs in Copenhagen and 95 percent in Stockholm. For Grotan BK, the corresponding rates were 20 percent and 74 percent. Forcide 78, KM 200, and Preventol D2 sensitized 20 percent to 25 percent of the animals in Copenhagen, while Gerwall 115 and Grotan OX sensitized 60 percent to 70 percent of the animals in Stockholm.

The compounds tested in Copenhagen were all classified as mild to moderate sensitizers. In Stockholm, Grotan BK, Grotan OX, and Gerwall 115 formaldehyde were classified as strong to extreme sensitizers.

This paper did not provide any details of the procedures used, including the prescreen to determine the doses to be used in the actual test, the individual animal body weights or sensitization scores.

The study is classified as Supplementary.

A Study of Biocides for the Selection of Candidates for Carcinogen Bioassay. O. H. Johnson, S. Casey, M. K. Doeltz, K.E. McCaleb, A. M. Miller, P.A. Papa, L. B. Swelt, M. A. Valentine and C. T. Helmes. J. Environ. Sci. Health A 19:1-25, 1984.

The test chemical, hexahydro-1,3,5-tris(2-hydroxy-ethyl)triazine, was among a preliminary list of 295 chemicals considered to be nominated as candidates for carcinogen bioassay to the National Cancer Institute (NCI). The nominations were made by the Chemical Selection Working Group (CSWG) an interagency group operating through the Office of the Scientific Coordinator for Environmental Cancer in the Division of Cancer Cause and Prevention (DCCP) at NCI. The selection of the biocides class for study was based on a consideration of both potential for human exposure and suspicion of possible carcinogenicity. Chemicals were eliminated from the preliminary list according to whether they had been tested for carcinogenicity, already reviewed as candidates for bioassay, commercially significant, or currently in use as biocides.

Two hundred thirty-four chemicals were then eliminated from further study, leaving 61 final candidate chemicals. The final candidate list was divided into structural subclasses in order to assign levels of suspicion of carcinogenic activity. Structural subclasses were established: alcohols, aliphatic acids and esters, aliphatic nitrogen-containing compounds, alkyl halides, aromatic amines and amides, aryl halides,

miscellaneous benzene ring-containing compounds, nitrogen heterocytes, organometals, and phenols. The 61 remaining chemicals were nominated for carcinogenicity for testing based on (1) their structural subclass, (2) previous positive oncogenicity in limited animal studies, (3) positive in vitro carcinogenic prescreens, and (4) theoretical reason e.g., an unusual structure or potential for nitrosation, presence of polar groups to facilitate detoxification, and excretion. Chemicals for which no meaningful data on carcinogenic potential were available, or no theoretical considerations were relevant, were considered "gaps in knowledge" and classified as of "unknown" suspicion.

Eight chemicals of the aliphatic nitrogen-containing groups were nominated. Hexahydro-1,3,5-tris(2 hydroxyethyl)triazine was nominated (Cas. No. 4719-04-4) on the basis of its considerable potential for human exposure and its unknown suspicion of carcinogenicity. United States estimated consumption in 1973 was 4.5×10^8 g. It is a major biocide used in soluble cutting oil emulsions.

Selection for Study

The selection decisions made by the CSWG and the CSSG are shown in the following table, taken from the literature report.

Selection Decisions for Nominated Chemicals

Chemical	CSWG Selected	CSWG Priority	CSWG Rejected	CSSG Priority
Benzethonium chloride	X*			
Hexahydro-1,3,5-tris(2-hydroxyethyl)triazine			X	5.5/10
Benzyldimethyl(mixed alkyl) ammonium chloride			X	
N-(3-Chloroallyl)hexaminium chloride	X	Moderate		5.0/10
2,4,5-Trichlorophenol	X	Moderate-high		6.4/10
o-Benzyl-p-chlorophenol	X	Moderate		3.1/10
1,2-Benzisothiazolin-3-one			X	
Zinc pyrithione			X	

* Selected for testing by skin painting.

Hexahydro-1,3,5-tris(2-hydroxyethyl)triazine was not recommended to NCI for study because of a lack of suspicion of carcinogencity.

Discussion

This report was included in the submission to be used as a basis for a request for waiver of data for carcinogenicity studies.

The information contained in this submission is classified as Supplementary.

Ames Test (O.E.C.D.) Product Glokill 77. Safe Pharm Laboratories, Ltd., Derby, England, September 11, 1984. Acc. No.260195

Materials and Methods

The mutagenicity of the test compound, Glokill 77, was evaluated by the plate-incorporation method of the bacterial reversion assay according to the technique described by Ames et al. and Garner et al. The assay was done in the presence and absence of metabolic activation from rat liver enzymes. Four histidine-requiring strains of Salmonella typhimurium were used in the study: TA 100 and TA 1535 sensitive to agents inducing base-pair substitutions; TA 98 and TA 1537 sensitive to agents inducing frame-shift mutations.

The bacteria were obtained from Ames (Dept. of Biochemistry, University of California, Berkeley California). Subcultures were prepared in nutrient broth obtained from Oxoid Ltd., incubated at 37 °C for approximately 18 hours. Overnight cultures yielding 10^8 to 10^9 bacteria per mL were used as the standard bacterial suspension.

The microsomal enzyme fraction from Arochlor 1254 pretreated animals was commercially obtained and contained 40 mg/mL of protein. Positive controls used in the study were M-methyl-N-nitro-N-nitrosoquanidine (MNNG) 2 ug/plate for TA 1535 and TA 100; 9 amino-acridine (9AA) 100 ug/plate for TA 1537; and 4 nitro-o-phenyldiamine (4NOPD) 10 ug/plate for TA 98. In addition

2 amino-anthracene 10 ug/plate was used as the reference mutagen with metabolic activation for all strains.

A preliminary cytotoxicity study was performed to select appropriate dosage levels for the main study. The test compound was diluted with deionized water, and 0.1 mL aliquots of concentrations ranging from 200, 1000, 5000, 25,000, 125,000 ug/mL were added to sterile plates containing 0.1 mL of bacterial suspension, histidine deficient media, and minimal agar. Plates were incubated at 37 °C for 24 hours prior to assessing for the toxicity of the test compound by measuring the inhibition zones produced.

Main Study

Five concentrations (0.16, 0.8, 4, 20, and 100 ug/plate) of the test material were assayed in triplicate against each tester strain, with and without metabolic activation. Solvent and positive controls were run with each assay.

Aliquots of the assay mixtures were poured on minimal agar plates, incubated for 48 hours at 37 °C and the number of revertant colonies counted. The complete experiment was repeated with fresh bacterial cultures, and new test and control solutions. The assays done with metabolic activation used 0.5 mL of the S-9 liver microsomal mix.

Evaluation Criteria

The test substance was considered mutagenic if it produced a dose-related and statistically significant increase in mutation

rates (of at least twice the spontaneous reversion rate) in one or more strains of bacteria in the presence and/or absence of the S-9 microsomal enzymes. Conversely, if the number of induced revertants compared to spontaneous revertants were less than twofold at all dose levels employed up to the limit of toxicity or solubility, the test compound was considered not to be mutagenic.

Results Reported

In the preliminary study, the test compound was toxic at all dosage levels used. Dosage levels of 0.16, 0.8, 4, 20, and 100 ug/plate were used in the main study based on the results of the preliminary study. No significant increase in the number of revertant colonies was reported for TA 100 and TA 1535 strains of Salmonella typhimurium; however, in strain TA 98 a significant increase in the number of revertant colonies was reported in the assay with metabolic activation, and in strain TA 1538 an increase in the number of revertant colonies was reported both with and without metabolic activation. All positive control substances produced significant increases in the number of revertant colonies. The spontaneous reversion rate for each strain was within the expected range. The histidine/biotin solution, vehicle, and S-9 mix were reported to be sterile.

Discussion and Conclusions

Salmonella typhimurium strain TA 1538 was not included in the Materials and Methods section of the report. The test compound

caused an increase in the mutation frequency of strain TA 98. In the first experiment, although the increase in mutation freq-quency was significantly increased over the spontaneous mutation frequency in untreated bacteria, the mean number of revertant colonies remained the same at all dosage levels in the study using metabolic activation.

At the highest dosage level, 100 ug/plate, the test compound appeared to be cytotoxic. In experiment 2, the mean number of revertant colonies doubled, but the two highest dose levels had lower mutation frequencies than the two middle dose; thus the mutation frequency does not seem to be dose related.

In strain TA 1538, in experiment 1 without metabolic activation, the mutation frequency was significantly increased in a dose-dependent manner up to the high dose (100 ug/plate) which appreared to be cytotoxic. In the assay with metabolic activation, the increase in revertant colonies did not appear to be dose related. The highest dose level in this assay appeared to be cytotoxic. In the second experiment, the two highest dosage levels produced a small increase in the number of revertant colonies when the assay was performed without metabolic activation. When the assay was done with metabolic activation the increase in the number of revertant colonies was dose related.

The results of the assays in strains TA 98 and TA 1538 of Salmonella typhimurium were not reproducible. Based on this, the results of the study seem to be inconclusive. The study should be repeated.

An Evaluation of the Mutagenicity of the Cutting Oil Preservative Grotan BK. C. Urwin, Julian C. Richardson, and Anthony K. Palmer. Mutation Research 40:43-46, 1976.

The micronucleus test in rats was used to investigate the mutagenic potential of Grotan BK, a preserving agent used in industrial cutting oils.

Materials and Methods

The test chemical was administered orally, dermally, or subcutaneously in 4 percent cutting oil/water emulsion at dosages of 7.5, 30, 120, and 480 mg/kg daily for 2 days to Specific Pathogen Free Wistar rats. Benzidine 204.8 mg/kg was the positive control, and was administered dermally and subcutaneously, because it is ineffective orally. The negative control animals were administered the 4 percent cutting oil vehicle only.

Six hours after administration of the second dose of the test compound, vehicle or benzidine, the rats were sacrificed by CO_2 asphixiation, and air-dried smears of bone marrow prepared according to the method of Schmid. For each rat 2000 polychromatic erythrocytes were examined in the bone-marrow smear, and the incidence of micronucleated red cells recorded.

Results

The incidence of micronucleated polychromatic erythrocytes was not increased with Grotan BK at any dose level or route of administration. A marked increase in micronucleated polychromatic erythrocytes was reported for the positive control groups treated with benzidine.

Discussion and Conclusions

Although negative results were reported in the study, it does not satisfy the Agency's requirement for an acceptable mutagenicity study because the report was not detailed enough for the study to be evaluated. Among the deficiencies noted are:

1. The age, number, and sex of animals in the control and treated groups was not stated.

2. Criteria for identifying micronucleated erythrocytes or for evaluating the results of the study were not given.

3. No individual animal data were supplied.

The study is Unacceptable.