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

003775

MEMORANDUM

MAR 23 1983

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: James Yowell, Project Manager
Special Pesticide Review Division

THRU: Orville E. Paynter, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769)

WL Burnam 3.22.83

SUBJECT: EPA#4581-116; Priority Review of Cryolite Toxicity Data (Registration Standard).
CASWELL#204 Accession No. 071392

Registrant: Pennwalt Corp.

Action Requested:

In order to expedite review of recently submitted Cryolite toxicity data provided by the Pennwalt Corporation in conjunction with Registration Standard data call-in and current data requirements, five Toxicology Branch reviewers contributed to this effort:

Dr. Teeters
Dr. Zendzian
Dr. Mauer
Dr. Woodrow
Mr. Burin

(See attached memo of March 11, 1983 by William L. Burnam, Deputy Chief, Toxicology Branch).

Recommendations:

Summary of Cryolite toxicity data submitted by the Pennwalt Corp.

A. Acute Toxicity Studies, reviewed by Woodrow

a) Acute Oral Toxicity of Kryocide, Rat
$LD_{50}$ > 1.5 g/kg
Toxicity Category III
Classification: Supplementary Data (In order to satisfy acute oral data requirements using one dose level, 5 g/kg, or more should have be tested).

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b) Primary Dermal Irritation Evaluation of Kryocide, Rabbit.
Not an irritant.
P.I. score = 0.0
Toxicity Category - IV
Classification: Core-Minimum Data

c) Primary Eye Irritation Evaluation of Kryocide, Rabbit.
Moderate conjunctival irritation that disappeared within 7 days.
Toxicity Category - III
Classification: Core-Minimum Data

d) Acute Dermal Toxicity Evaluation of Kryocide, Rabbit.
$LD_{50}$ (dermal) > 2.1 g/kg
Toxicity Category - III
Classification: Core-Minimum Data

e) Acute Inhalation Toxicity Evaluation of Kryocide, Rat.
$LC_{50}$ < 5.03 mg/L, > 2.06 mg/L (T.W.A.)
Toxicity Category - III
Classification: Core-Minimum Data

B. Metabolism Study reviewed by Dr. Zendzian. (No study classification was designated).

C. Twenty-eight Day Range-finding and Palatibility Study of Kryocide in Rats, reviewed by Dr. Teeters.

A NOEL was not established (color changes in incisors were seen at all test dose levels; changes in the continuously growing incisors were the only effects observed).

Classification: Core-Minimum Data

D. Genetic Studies reviewed by Dr. Mauer.

a) Salmonella/Microsomal Mutagenicity (Ames) Assay of Kryocide Technical.

Classifiction: Unacceptable (a repeat test was not performed, no toxicity testing was reported, and chemical characterization of the test material was not included).

b) DNA Repair Evaluation of Kryocide Technical.

Classification: Inconclusive (The highest dose may not have been used, solubility data were not provided, and chemical characterization of the test material was not included).

c) In Vivo Cytogenetic Evaluation of Kryocide Technical.

Classification: Unacceptable: Test chemical not characterized; solubility, nature and conc. of impurities not stated; it is possible that insufficient dosage may have been administered.

E. Range-finding and Primary Teratology Studies in Rats, Using Kryocide Technical, reviewed by Gary Burin.

No mortalities, the only effects found were whitening of rat tooth enamel.

It is recommended that this study be classified as Supplementary Data because a Final Report is not yet available. After the submission of a Final Report, it is likely that this study can be upgraded to Core-Minimum. The NOEL for maternal and fetotoxicity is 3000 mg/kg (aside from possible toxicity associated with whitening of the teeth of dams, noted in all animals). A teratogenic potential for Cryolite is not indicated in this study.

1) Review by W. Woodrow (Acute Toxicity Studies).

a. Acute Oral Toxicity Evaluation of Kryocide, Rat. Sponsor: Pennwalt Corp. Tester: Raltech Scientific Services. #880531, September 21, 1981.

Test Material: Synthetic Cryolite (Kryocide), 96% pure. ($Na_3AlF_6$)

Five male and five female Sprague-Dawley rats were acclimated to laboratory conditions for 7 days. Prior to dosing, food only was withheld overnight. All 10 animals were dosed by gavage with 1.5 g/kg body weight of the test Kryocide prepared by grinding in a mortar and pestle with distilled water. The dose volume of test mixture was 10.0 ml/kg of body weight.

Animals were observed for pharmacotoxic signs and mortality at 1, 2.5, 4 hours, and twice per day through 14 days following test compound administration. Animals were weighed prior to testing, and body weights of survivors were measured again at 7 and 14 days post treatment.

At study termination, surviving animals were killed and subjected to a gross necropsy examination.

Results:

No mortality. Body weight increases were recorded for both male and female rats. One male rat exhibited a reddened thymus gland at necropsy. One female was found to contain a moderate hydromatous uterus, and a second female exhibited a mild hydromatous uterus. These were the only remarkable necropsy findings.

$LD_{50}$ > 1.5 g/kg body weight.

Toxicity Category: III

Classification: Supplementary Data (In order to satisfy acute oral data requirements using one dose level, the animals should have been dosed with 5 g/kg body weight).

b. Primary Dermal Irritation Evaluation of Kryocide, Rabbit. Sponsor: Pennwalt Corp. Tester: Raltech Scientific Services. #880531, September 21, 1981.

Test Material: Kryocide (Synthetic Cryolite) $Na_3AlF_6$, 96% pure. The test material was moistened and applied as received by the testing lab.

One-half (0.5 g) gram of moistened test material was applied to two abraded and two intact skin sites on each of three male and three female NZW rabbits. The animals had been acclimated to laboratory conditions for a period of 7 days prior to testing. The backs and flanks of the animals were clipped to remove hair prior to test application. Five x5 cm gauze patches were used to maintain the test material; etastoplast tape was then applied to the protected test sites. Collars were fitted to the animals during the 24-hour treatment period.

Twenty four hours following application, treated skin sites were uncovered, wiped, and the degree of edema and erythema was recorded according to the Draize system of scoring. A second reading was made at 72 hours.

Results: Primary irritation score = 0.0; Kryocide did not demonstrate any irritation potential in this experiment.

Toxicity Category: IV

Classification: Core-Minimum Data

c. Primary Eye Irritation Evaluation of Kryocide, Rabbit. Sponsor: Pennwalt Corp. Tester: Raltech Scientific Services. #880531, September 21, 1981.

Test Material: Finely ground Kryocide (Cryolite - $Na_3AlF_6$), 96% pure.

Nine young NZW rabbits were acclimated to laboratory conditions for a period of 7 days before testing. The animals eyes were examined using fluorescein dye at least 24 hours before administering the test material, to screen for prior corneal injury. One-tenth gram of test material was placed on the everted lower lid of one eye of each of the nine rabbits. Upper and lower lids of treated eyes were briefly closed by hand for 1 second and released. Untreated eyes of each animal served as controls. The treated eyes of 3 of the rabbits were flushed for one minute with water starting 30 seconds after test compound administration; treated eyes of the remaining 6 rabbits were not flushed with water.

The treated eyes of all test rabbits were observed for ocular lesions at 24, 48, 72, and 96 hours and at 7 days post treatment. At the 72 hour and again at the 7 day eye examinations, sodium fluorescein and ultraviolet light were employed to detect possible corneal injury. Ocular examination scoring was done according to Draize.

Results:

| Irritation Scores | Unwashed Eyes | Washed Eyes |
|---|---|---|
| 24 hours | 5.3 | 3.7 |
| 48 hours | 2.7 | 2.0 |
| 72 hours | 0.7 | 1.3 |
| 96 hours | 0.5 | 0.0 |
| 7 days | 0.0 | 0.0 |

No corneal involvement; moderate conjunctival redness and chemosis irritation that disappeared within 7 days post treatment.

Toxicity Category: III

Classification: Core-Minium Data

d. Acute Dermal Toxicity Evaluation of Kryocide, Rabbits. Sponsor: Pennwalt Corp. Tester: ELARS Bioresearch Laboratories. #1685-6, July 20, 1981.

Test Material: Kryocide (Cryolite), fine white powder (96% pure).

Twenty NZW rabbits (10 males and 10 females) approximately 2 months old were acclimiated to laboratory conditions for 18 days prior to testing. The rabbits weighed 2.2 to 3.3 kg at initiation of the study

Twenty four hours before dermal treatment, approximately 10% of the animals backs and flanks were clipped free of hair.

On the day of testing prior to application, all rabbits were weighed to determine dosages, and the exposure sites on all rabbits were abraded.

Five male and five female rabbits received 2.1 g/kg body weight of Kryocide, moistened with physiological saline applied under 4" x 4" gauze sponges which were held in place with plastic wrap. The sponges and plastic wrap were taped to the shaved treatment sites, and then the animals trunks were wrapped with elastic tape.

Five male and five female rabbits were similarly treated with physiological saline only, to serve as controls.

Twenty hours following compound application, the treated test sites were uncovered and wiped to remove excess test material. The animals were observed for mortality, skin irritation and behavioral abnormalities twice daily for a total of 14 days. Body weights were recorded at 0, 7 and at termination (14 days). All rabbits were killed and subjected to gross necropsy; skin samples at the test sites were collected for histopathological examination.

Results:

No mortality. All rabbits treated with Kryocide displayed slightly red and swollen skin at test sites. Most of the rabbits appeared normal throughout the entire test period; however, occasional soft stool, diarrhea, or ocular discharge was noted in several of the treated animals. The control rabbits appeared normal.

One of the rabbits treated with Kryocide had pale, pitted kidneys and another in the same group had enlarged mesenteric lymph nodes. One control rabbit had diarrhea and one other had a slightly enlarged spleen.

Histopathologic examination revealed that eight of ten test group animals showed very slight to slight acanthosis, fibrosis, hyperkeratosis, and chronic dermal inflammation at the test sites. Dermal lesions were not found in the skin of control animals.

Dermal $LD_{50}$ = > 2.1 g/kg body weight.

Toxicity Category: III

Classification: Core-Minimum Data

e. Acute Inhalation Toxicity Evaluation of Kryocide, Rats. Sponsor: Pennwalt Corp. Tester: Litton Bionetics. LBI#22098, June, 1981.

Test Material: Kryocide (Cryolite), white powder, 96% pure.

This test was actually conducted in two parts; apparently the first (Expt. #1) inhalation study (April 8, 1981) test material concentration killed 90% of the test rats within two days post treatment. Therefore a second inhalation exposure (Expt. #2) was conducted on May 21, 1981, using a reduced inhalation exposure level.

One series of particle size measurements were made using an Anderson cascade Impactor during the two different experiments; presumably prior to the first exposure, April 8, 1981.

Five male and five female Charles River CD strain rats weighing between 192.7 and 269.1 g were acclimated to laboratory conditions for 7 days prior to Expt. #1 testing.

Five male and five female Charles River CD strain rats that weighed between 255.4 to 423.8 g were acclimated to laboratory conditions for 6 weeks prior to testing in Expt. #2.

The exposure cloud concentrations were generated by blowing dry filtered air through a fluid bed generator into the exposure chamber. The exposure chamber for both inhalation tests consisted of a 30 liter glass cylinder.

The exposed rats were observed frequently on the day of exposure and twice daily for toxic signs and mortality throughout the 14 day observation period. Test animals were weighed on days 0, 2, 3, 4, 7, and 14 post exposure. Necropsies were performed on all animals that died during exposure and on survivors at experiment termination.

Results:

a. Mortality: Group 1 (5.03 mg/L T.W.A.*)

| | Day of Death |
|---|---|
| 5/5 males | 1 |
| 4/5 females | 2 |

Group 2 (2.06 mg/L T.W.A.)

0/5 males
0/5 females

*Time Weighted Average

The actual cloud concentrations were determined on an hourly basis by withdrawing cloud samples (during the 4 hour exposure periods) through filters in measured volumes. The gain in filter weight was divided by sample volume to determine actual concentrations per liter of exposure chamber atmosphere (telecon communication between Woodrow and P.J. Knapinski of Litton Bionetics).

Results (continued):

Mortality: Nine of 10 animals exposed to T.W.A. of 5.03 mg/L (group 1) died by day 2 post exposure. One survivor was sacrificed at termination (14 days).

All 10 animals in group 2 exposed to T.W.A. 2.06 mg/L survived to the experiment termination.

Body Weight: The one female survivor in group one, and all of the test animals in group 2 lost body weight until approximately 7 days post exposure, and thereafter began to gain weight.

Toxic Signs: Group 1 animals (5.03 mg/L) displayed labored breathing, abnormal respiratory sounds, slowed righting reflex, slow movement, and lacrimation in two of the group animals.

One male animal in group 2 (2.06 mg/L) exhibited abnormal respiratory sounds, and one other group 2 male displayed a slowed righting reflex.

Necropsy: All of the rats that died exhibited red lungs with pale mottling; the animals that survived to termination at 14 days did not show these signs. The survivors did show varying degrees of mottling.

$LC_{50}$: < 5.03 mg/L, > 2.06 mg/L (T.W.A.)

Toxicity Category: III

Classification: Core-Minimum Data

2) Review by Dr. Zendzian.

Metabolism

A metabolism study of cryolite (Kryocide®) is not required. The metabolism of the ionic components of this inorganic compound are sufficiently established to satisfy the needs of the agency.

Cryolite ($Na_3AlF_6$) is the active ingredient of Kryocide® Insecticide. The compound has a low solubility in water solutions producing ionic sodium, aluminum and floride. These ions are the only portion of the compound that can be biologically absorbed. Sodium ion is a normal constituant of the body and is regularly handled in relatively large amounts by the organism. It is of no toxicological concern. The aluminum ion is found in small quantities in the body but is not known if it has any biological function. Aluminum poisoning had been established in kidney dialysis where it enters the blood directly from the dialysis water. However it is only poorly absorbed orally and is not generally considered a toxic metal. The floride ion is rapidly absorbed and handled biologically in the same fashion as the chloride ion. Its toxicity is due to its being an inappropriate substitute for chloride ion in various electochemical processes in the organism, generally a part of the process of electrical excitability of cells. The fluoride ion can become part of bone where it is toxicologically inert. Floride ion is rapidly excreted by the kidney in preference to the chloride ion. Bioaccumulation does not occur and when the oral intake of floride ceases body concentrations fall rapidly. The low solubility of cryolite further limits the amount of fluoride ion available for absorption.

The metabolism of cryolite (an inorganic compound) is a function of its ions. The metabolism of these ions is sufficiently understood to satisfy the requirements of pesticide registration, metabolism studies of cryolite are not required.

The report Kryocide® Insecticide Absorption in the Rat Project No. WT-12-82 2/1/83 submitted by the registrant, Pennwalt, clearly shows that the established body of information on floride metabolism is correct for cryolite. Floride ion is only slightly absorbed and is rapidly and completely excreted in the urine.

3) Review by Dr. Winnie Teeters (Twenty-eight Day Range-finding and Palatability Study with Kryocide).

Twenty-eight Day Range Finding and Palatability Study With KRYOCIDE® Insecticide in Rats.

Elars Bioresearch Laboratories, Inc.
Project NO. 1821, Final Report Date: 6-29-82
Histology done by Westpath Laboratories, Inc.
Project No. 1242

Review:

Procedure: After two weeks of acclimation, 60 weanling Sprague-Dawley albino rats were divided into groups containing 5/sex/group and fed the following dietary levels of KRYOCIDE insecticide: 0, 250, 500, 1000, 2000 or 4000 ppm for 28 days. An additional similar group was started on Day 15 at a level of 10,000 ppm. These animals were from the same shipment as the other groups and had been maintained in the same room, but fed untreated diet. Housing was individual and food and water were available ad libitum. Three control rats/sex were kept on the study for sacrifice with the added group. These 7 groups constituted Phase I.

Four more additional groups of 5 rats/sex/group were fed levels of 0, 10,000, 25,000 or 50,000 ppm of KRYOCIDE for 28 days, when no effects were seen in Phase I. These four levels constituted Phase II.

Lot Number NB#86-11-9 of KRYOCIDE insecticide was used; the active ingredient is sodium fluoaluminate (97.6%). Stability data of the test material in diet mixtures were determined and water was analyzed quarterly to check quality and assure less than 2 ppm fluoride content.

Observations were made twice daily for signs of toxicity, pharmacological effects, behavioral abnormalities, moribundity and mortality. Gross examinations of the teeth were conducted periodically.

Body weights and food consumption were determined weekly. Determinations for blood urea nitrogen, serum alkaline phosphatase, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, creatinine, calcium and phosphorus were made on rats of Phase II after 28 days on study.

All animals were necropsied; the examination included the external surface, all orifices, cranial cavity, carcass, external and cut surfaces of the brain and spinal cord, thoracic cavity and viscera, and abdominal cavity and viscera. Liver weights were determined for all rats and liver-to-body weight ratios were calculated. Sections of liver and kidneys were preserved. The entire animal was preserved for all Phase II rats, and additional tissues, mostly bone and teeth, from rats in the control and 50,000 ppm groups were selected for microscopic examination.

Group means for clinical chemistry parameters, body weight, food consumption and organ weights and ratios were calculated and analyzed statistically for group differences (ANOVA followed by Tukey's HSD).

Results:

Males in the 10,000 ppm group of Phase I weighed slightly less than controls throughout the four weeks; but in Phase II, when this dose was repeated, male weights were similar to controls at this level as well as higher levels. As found for the males, the females at 10,000 ppm also weighed less than controls during Phase I. This effect was also present for this sex and dose level in Phase II and, in fact, for all levels for this sex throughout Phase II, but the effect was not related to dose and was maximally only about a 12% decrease.

Mean food consumption of males at 10,000 ppm was consistently less than controls during Phase I, but was similar to controls during Phase II for this and all higher levels. Similarly, females of the highest level (10,000 ppm) consumed less food than controls during Phase I (statistically significantly less for their third week). During Phase II, food consumption was consistently less than controls for all treated females, but the differences were small (10% and less) and not significant nor related to dose.

Something happened during week 4 of Phase II to decrease food consumption (up to 39 g, mean) and body weights (up to 17.2 g mean) consistently for both sexes in all groups; this is uncommon for actively growing animals.

No mortalities occurred. It was stated that clinical signs (not defined) other than those noted for teeth were temporary and equally distributed among control and treated groups. Compound related effects on the teeth were limited to the incisors and were both time- and dose-related. These teeth changed from the orange-yellow color of normal rats to a white color and the enamel became soft and granular. Changes in color were more prevalent in lower incisors but changes in physical property of the enamel seemed restricted to the upper incisors, which occasionally were shorter than normal, possibly reflecting a propensity toward increased wear. In Phase I, teeth color changes were noted on

Day 14 for the 10,000 ppm level and were seen in all levels by Day 28, when 2/10 in the lowest level (250 ppm) had a color change; a physical change in enamel was seen in 2/10 at the next higher level (500 ppm) on Day 18. In Phase II, teeth changes were noted by Day 6 in all treated groups (10,000-50,000 ppm).

Statistically significant increases were found in Phase II for phosphorus of males (25,000 ppm) and phosphatase of females (50,000 ppm) and a decrease in creatinine of females (25,000 ppm). Although not significant, males showed a dose-dependent increase in phosphatase values (93.6, 95.8, 106.4 and 110 IU/L for control, low, mid and high levels, respectively), but for females all levels were similar to control except the highest one, which was significantly increased (64.2, 63.2, 62.6 and 99.2 IU/L for control, low, mid and high levels, respectively). All other mean clinical chemistry values were similar among the groups.

The only gross pathology findings, other than dose-related changes in the teeth, were a few isolated ones which did not appear to indicate adverse effects attributable to KRYOCIDE.

Although absolute liver weights and ratios were not statistically significantly different from controls for any group of either phase, both weights and ratios for both sexes in 25,000 and 50,000 ppm groups were consistently less than control.

The selected tissues examined microscopically from Phase II rats were: 1) both kidneys and the liver of each rat; 2) the tarsal bone of the right hind leg; a femur and tibia; 3 coronal sections of the nasal cavity including incisive, frontal, maxillar and other associated osseous structures within or peripheral to the nasal cavity; 2 cross-sections of the mandible, and any teeth associated with these cranial sections from all rats in the control and high level groups; and 3) approximately 40 tissues/rat from one rat of each sex in the high level group.

The only histologic findings in the limited tissues examined were murine nephropathy in both kidneys of one male (25,000 ppm) and atelectasis and lymphoid hyperplasia of the lungs of a rat of each sex exposed to 50,000 ppm.

It was stated (Westpath Lab. Inc. report, page 3) that radiological examination of the head and various segments of the right hind leg did not reveal any recognizable differences between control and treated rats. (Radiographs, provided only with the original report copy, were not available to this reviewer).

Core Classification:

This study is classified Core-Minimum.

The only compound-related effect was a change in coloration and physical property (soft and granular enamel) of the incisor teeth. The change in color was noted at all tested levels (250, 500, 1000, 2000, 4000, 10,000, 25,000 and 50,000 ppm) whereas a change in physical property was seen only at levels of 500 ppm and higher.

A NOEL was not established in this 28-day study since color changes in the incisors were seen at all levels tested, and with increased exposure duration probably would be followed by a physical change in the enamel. Yet changes in the continuously growing incisors were the only effects seen and their significance and the possibility of associated changes in body mineral metabolism and distribution will have to be investigated in longer term studies.

4) Review by Dr. Irving Mauer (Genetic Studies).

Kryocide(™) (Cryolite; sodium fluoaluminate) Caswell No. 264

Test Article: "T1693 (sodium fluoaluminate, Pennwalt), 96%, a white powder", dissolved in DMSO for assay. [Although not stated, presumably this is the technical chemical in Kryocide.]

a. Citation: "ACTIVITY OF KRYOCIDE IN THE SALMONELLA/MICROSOMAI ASSAY FOR BACTERIAL MUTAGENICITY. FINAL REPORT. "MICROBIOLOGICAL ASSOCIATES, Bethesda, Maryland, Report No. T1693.102, September 29, 1981; sponsored by Pennwalt Corporation, Tacoma, Washington. ["Exhibit 10" of cryolite RS]

Reason for Review: Cryolite Registration Standard.

Trade Secret Claim: CBI

Reviewed By: Irving Mauer, Ph.D.
Toxicology Pranch/HED

Date of Review: March 14, 1983

Test Type: Mutagencity - Reverse mutation in the Salmonella/Microsome Assay.

Study: Five histidine-requiring (his-) strains (Ames) of S. typhimurium were exposed to 5 concentrations of test article, ranging from 0.05 to 5.0 mg/plate diluted in DMSO (each dose in triplicate), both in the absence and presence of a metabolic activation system (MA) consisting of the microsomal fraction (S-9) prepared from Aroclor-induced rat liver, plus cofactors, according to standardized procedures. Negative controls, (DMSO solvent, 100 ml/plate; sterility, and S-9), as well as positive substances (the mutagens; sodium azide, 30 ug, for TA1535 and TA100; 9-aminoacridine, 10 ug, for TA1537; 2-nitrofluorene, 5 ug, for TA1538 and TA98 - in non-activated cultures; 2-aminoanthracene, 5.0 ug, for all 5 strains with MA) were included in each test. After 48 hours incubation with test articles, the number of visible revertent colonies were enumerated automatically, and corrected for masked and overlapping colonies greater than 12 (to reflect manual counts) by the following formula:

$$Y = 7.4 + 1.606x - (7.1 \times 10^{-4})X^2 + (6.31 \times 10^{-7})X^3$$

Where: Y = corrected counts, and X = machine counts. All machine counts greater than 1933, i.e. adjusted counts greater than 5000, were reported as to numerous to count (TNTC).

The mean (± s.d.) number of revertant colonies from each set of triplicate test compound plates was compared to that found with the solvent control. A "positive" (i.e., mutagenic) response was one where a dose related increase in the number of revertent colonies was found in the treatment groups, with the first dose level considered for the increase having an average number of revertent colonies three times that of the solvent control.

Tabulations of test results revealed that no concentration of cryolite induced as average number of revertants/plate three times greater than that found in the solvent control, either with or without MA, in contrast to expected responses of all positive controls (approx. 10 to 100 X DMSO values).

EVALUATION: Although generally conducted according to accepted protocols and controls, this study is incomplete, and hence UNACCEPTABLE as a comprehensive assay for mutagenicity in this bacterial system, due to the following deficiencies:

1) A repeat separate test was not performed (to confirm the "negative").

2) No toxicity testing was reported (survival data), hence it is uncertain that a sufficient dose was employed to be effective.

3) Chemical characterization of the test article was not included (nature of substance, solubilities, concentration of impurities and contaminants, etc.).

b. Citation: "ACTIVITY OF T1693 IN A DNA REPAIR TESTING USING ESCHERICHIA COLI. FINAL REPORT." "MICROBIOLOGICAL ASSOCIATES Bethesda, Maryland Report No. T1693.104, September 21, 1981; [Exhibit No. 11 of Cryolite RS].

Trade Secret Claim: CBI

Reviewed By: Irving Mauer, Ph.D.
Toxicology Branch/HED

Date of Review: March 15, 1983

Reason for Review: Cryolite Registration Standard

Test Type: Mutagenicity - DNA Damage (differential toxicity)/repair assay in Escherichia coli strains.

Study:

Differential zones of growth inhibition were to be compared in two strains of E. coli, P3478 (repair-deficient in polymerase I, i.e., pol $A^-$) and W3110 (normal for repair, i.e., pol $A^+$), exposed overnight to paper discs impregnated with 1.0, 0.3 or 0.1 ug test article, both in the absence and presence of a metabolic activation system (MA) consisting of the microsomal function (S-9) prepared to Aroclor-induced rat liver, plus cofactors, according to standardized procedures. Negative controls, (DMSO, and chloramphenicol, 30 ug), as well as 10 ul/disc positive substances [the mutagens, methylmethansulfonate (MMS) in non-activated cultures, and diethylnitrosamine (DEN) in MA tests were included in the assay.

Since no inhibition of growth was observed in any culture treated with cryolite, in contrast to expected responses of both types of control, the assay was considered inconclusive for DNA damage/repair under the conditions employed for this assay (i.e., "no test") [This may have been due to inactivity, of the test article, its non-diffusion, or incapacity to penetrate bacterial cells.]

Evaluation: The protocol employed in this study would be generally sufficient for this type of assay, except that the conclusion ("no test") is compromised by the following deficiencies.

1. The highest dose may not have been used.

2. Solubility data were not provided.

3. Chemical characterization of test article was not included (nature of substance, solubilities, concentration of impurities and contaminants, etc.).

Hence the study is INCONCLUSIVE and the author's conclusion not adequately documented.

c. Citation: "ACTIVITY OF T1693 IN THE IN VIVO CYTOGENETICS ASSAY IN RODENTS. FINAL REPORT. Report No. T1693.112, October 2, 1981, "MICROBIOLOGICAL ASSOCIATES, Bethesda, Maryland, sponsored by Pennwalt Corporation, Tacoma, Washington. [Exhibit 12 of Cryolite RS.].

Trade Secret Claim: CBI

Reason for Review: Cryolite Registration Standard.

Reviewed By: Irving Mauer, Ph.D.
Toxicology Branch/HED

Date of Review: March 15, 1983

Test Type: Rodent in vivo cytogenetics.

Study:

Following a range-finding study, juvenile male Sprague-Dawley rats (230-260 g) were dosed by gavage (12 ml/kg) at three levels of test article (in corn oil): 0.6, 1.8 and 6.0 g/kg daily for five days; femurs removed 6 hours after the last dose (colchicine, 4 mg/kg i.p. 2 hr. prior to sacrifice), and bone marrow cells prepared for microscopic cytogenetic screening by conventional methods. Fifty metaphases per animal on Giemsa stained slide preparations were scored for structural chromosome damage (breaks, fragmentation, rearrangements etc., including gaps) as well as for numerical aberrations (ploidies), and mitotic indices recorded for each rat (number of mitotic cells per 100 cells observed). Triethylenemelamine (TEM, 0.5 mg/kg once, one day prior to sacrifice) was used as the positive control. The carrier vehicle for the test article (corn oil) was used as the negative control at a dose level of 12 ml/kg/day.

Results were tabulated for individual and total aberrations per cell (expressed as percentage) as well as for severity of damage (aberrations per cell). Chromosome and chromatid gaps (usually defined as aligned, non-staining areas equal to or less than the width of the chromosomal element) were presented but not included in aberration scores. Statistical treatment of the data was by chi-square analysis of 2 x 2 contingency tables: damage vs no-damage classified against treatment vs control, relative to negative control group, for percentage aberrations; and pairwise one-sided t-test for severity.

The reported results and tabulations indicated no changes among treated rats at any dosage compared to corn oil controls in chromosome number (all = 42) or mitotic indices (3-6% vs 4-5%), and only one structural aberration (a chromatid break) was observed in the low-dose (0.6 g/kg) group. TEM treatment produced the expected response, severe damage in over 12% of cells (3/4 of which contained more than 10 aberrations each). [NB: A dose-related incidence and severity of chromatid and chromosome gaps is recorded in Tables IV and V.]

The authors concluded that "... no detectable clastogenic activity was found from test article T1693 when the in vivo cytogenetics assay was performed as described in this report."

Evaluation: This study is UNACCEPTABLE as a comprehensive assay for the chromosome-breaking potential of cryolite, because of the following deficiencies.

1. Test chemical was not characterized as to solubilities nature and concentrations of impurities and/or contaminants.

2. It is not clear that dose-selection by subchronic toxicity test was made on the same strain of rats as used in the assay.

3. No demonstrable toxic effects at the highest dosage were reported; only absence of weight gain.

4. Hence, insufficient dosage may have administered (less than MTD). [The reported acute oral $LD_{50}$ in rats appears to have been administered (10 g/kg, as given in Farm Chemicals Handbook), judging by the results of the preliminary range-finding reported, where death rates were recorded as 4 of 5 at 12 g/kg; 3 of 5 at 9 g/kg; and no deaths at 6 or 3 g/kg.

5. Although the biological significance of structural "gaps" is uncertain, dose-related increases commonly accompany evidence of frank chromosome damage.

6. No assurance was given that the test article was absorbed from the g.i. tract (no effects reported on cell toxicity, e.g.).

7. Females were not tested.

5) Reviewed by Gary Burin (Teratology Studies).

Review of Data:

Range-finding and Primary Teratology Studies, Rats. Conducted at Science Applications, Inc., La Jolla, CA, draft Final Report January 5, 1983, study nos. 1182007 and 1182008.

a. Range-finding Phase

Sprague-Dawley derived FBR Simonsen albino rats were administered Kryocide technical in a 0.2% carboxymethyl cellulose aqueous suspension on days 6-19 of gestation. Six dams per dose level were treated at levels of 0, 750, 1500 and 3000 mg/kg and 5 dams were treated at 375 mg/kg. All dams were weighed and observed daily. On the 20th day of gestation, laparohysterectomies were conducted on all dams, uteruses examined, and fetuses weighed, sexed and examined externally.

Results:

One dam from the 3000 mg/kg group died on day 18 of gestation; no other mortalities occurred. No clinical signs of toxicity were observed. Gross necropsy revealed pinpoint hemorrhages in 3 of 6 dams at the high dose level, including the dam that died. Body weights were similar in all groups.

The mean number of implantations per litter, the % of live fetuses, mean number of live fetuses per litter and the mean fetal weights were similar among all groups. The only recorded external observation was one fetus classified as a runt in the 1500 mg/kg group.

In summary, neither maternal nor fetoxicity was observed at dose levels up to and including 3000 mg/kg in this range-finding study.

b. Primary Study (Draft Report)

Note: A Final Report for this study has not yet been submitted. The following study evaluation is based on the draft Final Report of January 5, 1983.

Sprague-Dawley derived fBR Simonsen albino rats were administered Kryocide technical by gavage in a 0.2% carboxymethyl cellulose aqueous suspension on days 6-19 of gestation. Thirty dams per dose level were treated at dose levels of 0, 750, 1500 and 3000 mg/kg. Animals were weighed on days 0 and 6-19 of gestation and prior to sacrifice on day 20. Animals were sacrificed on day 20 of gestation by $CO_2$ asphyxiation.

Dams were necropsied and the thoracic and abdominal cavities examined in situ. The ovaries and uterus were examined and the number of corpora lutea, location and distribution of live and dead fetuses and number any type of resorption sites recorded. Each fetus was weighed, sexed and examined externally.

One half of the fetuses from each litter were decapitated and the fetal heads fixed in Bouin's solution for one week. The heads were then cross-sectioned using the technique of Wilson.

Each fetus was viscerally examined by dissection of the abdominal and thoracic cavities. Fetuses were then fixed, stained and examined for skeletal abnormalities.

Results:

No maternal mortalities were observed during the course of the study. The only treatment related maternal effect appeared to be whitening of the tooth enamel, observed in all treated animals. Body weights were similar in all groups. The distribution of gross lesions was similar in all groups. No treatment-related effects on litter size, mean fetal weight, resorption rate, number of implantation/litter or sex ratio were observed.

External malformations consisted of occasional runting and a single instance of umbilical cord hernia in the low dose group. Slight hydroureter was observed in a single fetus of the mid dose group. A number of skeletal variations and malformations were observed; however, their distribution was similar between the control and treated groups.

Core Classification: Supplementary Data

A Final Report for this study had not yet seen submitted. After it's submission, it is likely that this study can be upgraded to Core Minimum. The NOEL for maternal and fetotoxicity are 3000 mg/kg (aside from possible toxicity associated with whitening of the teeth of dams, noted in all animals). A teratogenic potential for Kryocide is not indicated in this study.

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