

UNDATED

in file

Bacteriostat CH 3565

60:955

Acute Oral Toxicity (Rat)

Young albino rats of the Sprague-Dawley strain with a body weight range from 200 to 225 grams were used as a test animals. The animals were housed in stock cages and permitted a standard laboratory rat diet plus water, ad libitum until 16 hours immediately prior to oral intubation.

After a 16-hour fast, selected dose groups (3.0 gm/Kg, 4.6 gm/Kg, 6.8 gm/Kg) of ten rats each (five male and five female) were intubated with the calculated doses of the test material in the form of a 10.0 per cent (w/v) corn oil suspension. All doses were administered directly into the stomachs of the rats using a hypodermic syringe equipped with a ball-pointed intubating needle.

Following oral administration of the test material, the rats were housed individually in observation cages and observed for the succeeding 14 days. Initial and final body weight as well as all mortalities and/or reactions displayed were recorded.

Results

The acute oral toxicity for male albino rats calculated to be 4.4 g/Kg with a standard deviation of $LD_{50} = \pm 0.4$ g/Kg.

The acute oral toxicity for female albino rats calculated 3.7 g/Kg with a standard deviation of $LD_{50} = \pm 0.5$ g/Kg. The reactions at all dose levels consisted of hypoactivity, diarrhea, diuresis and bloody nasal discharge during the first six hours. With the exception of bloody nasal discharge, these reactions plus muscular weakness and ruffed fur were also noted at the 22-hour point among surviving animals. These reactions continued for three to ten days or until death intervened. Death occurred between six and 96 hours following dose administration. Necropsy of animals which died during the study as well as of those sacrificed at the end of the 14-day observation period did not reveal any significant gross

BEST AVAILABLE COPY

1915

001955

pathologic alterations in the tissues and organs examined.

Acute Oral Toxicity (Mouse)

Five mice (male and female) were used in each dosage level of 2500 mg/Kg and 5000 mg/Kg. The material was administered in a suspension in gum arabic at 20% concentration. A single oral administration by gastric tube. The animals were observed for eight days after administration of the material. The LD₅₀ was calculated by interpolation on a probability graph.

Results

There was 0% mortality at the 2500 mg/Kg dosage range. At the 5000 mg/Kg dosage range there was an 80% mortality. The LD₅₀ calculated to be 4530 mg/Kg.

Acute Oral Toxicity (Dog)

Two test groups, each consisting of two male and two female dogs and corresponding to dose levels of 2500 to 5000 mg/Kg respectively employed in the investigation. On the morning of the first test day, after a 16-hour fast (water permitted) all dogs received their respective doses of the undiluted test material via gelatin capsule, following oral administration of the test material, the dogs were observed for the succeeding 14 days. All mortalities and/or reactions displayed were recorded.

Results

Emesis occurred among all animals at both dosage levels within four hours after dosing. This reaction subsided within eleven hours after dosage and all animals appeared normal for the remainder of the study. No other reactions were noted at any time during the investigation.

Subacute Toxicity Oral (Rat)

Ten rats (half males, half females) were used in this study at each dosage level. The dosage levels employed were 50, 100, 200, 500, and 1000 mg/Kg daily. The

BEST AVAILABLE COPY

3

001955

compound was administered by gastric tube once daily over a period of four weeks (except Sundays). The volume administered was 10 cc/Kg. The Hematology and urinalysis plus micropathology were performed at the termination of the experiment.

Results

No untoward reactions were noted in the test groups receiving 50 - 500 mg/Kg.

Six out of ten animals died within five to fifteen days in the 1000 mg/Kg group.

Acute Dust Inhalation Toxicity (Rats)

Young albino rats having an average body weight of 250 grams were employed as test animals. Ten rats (five male and five female) were selected from the study. The exposure ran for a four-hour period during which time observations were made as to the incidence of mortality and reactions displayed. At the end of the exposure period, the rats were returned to their stock cages and observed for the following 14 days.

Body weights were determined for each animal prior to inhalation exposure and for each surviving animal at the end of the 14-day observation period. Gross pathologic examinations were scheduled to be conducted upon all animals which might succumb during the test as well as those sacrificed at the end of the test period.

The inhalation chamber was specially constructed all-Plexiglas having a volume of 70 liters. The chamber was designed so that the animals could be introduced to the test atmosphere after the optimum dust concentration was established. Each animal was caged separately during exposure. Dust was suspended by a specially designed dust feeder periodically capable of producing high concentrations over a long period of time. The concentration of test material in the exposure chamber was determined by sampling the test atmosphere in the breathing zone of the animals. Airborne particulates were collected on a tared glass fiber filter. The average

BEST AVAILABLE COPY

3

4
001955

dust concentration, as determined by repeated air sampling, was 0.14 mg/L of air at 29.9 inches Hg and 23°C. Sixty-Five per cent of the particles collected range in size from between one and five microns.

Results

No deaths or untoward behavioral reactions were observed among any test animals exposed four hours at an average concentration of 0.14 mg/L air. No adverse body weight effects were noted among any test animals. No gross pathologic findings or alterations in the tissues and organs examined were observed.

Chronic Toxicity (18-Month Study of the Carcinogenic Potential of Bacteriostat CH 3565 Mice)

An 18-month carcinogenic study was initiated January 3, 1967 in Swiss White Mice. The study was organized as follows: There were ten test groups; each consisting of 50 mice (half male, half female). Test groups one, three, five, seven, and ten received 0.1 ml of 0.5 per cent of the solution of bacteriostat 3565 in acetone. Test groups two, four, six, and nine each received 0.1 ml of a one per cent solution of bacteriostat 3565 in acetone. There was one positive control group consisting of 100 mice (half male and half female) which received 0.1 ml of a 0.1% solution of 9, 10, Demethyl 1, 2, Benzanthracene in acetone. One treated control group consisting of 100 animals (half male, and half female) which received 0.1 ml of undiluted acetone. One untreated controlled group consisting of 100 animals (half male and half female) which received no treatment. The material was applied dermally to the same shaved area (1 cm²) in the interscapular region. Three applications were made weekly.

Results

(6-month n/m) The body weights recorded for the first six months were testing revealed a normal group pattern for all animals tested. Food consumption measurement recorded during the first six months revealed normal food consumption of all animals tested. The mortalities of the test groups and of the treated controls were

4

001955

consistent with the mortalities of the untreated control group during the first 6-months of the study. The mortalities of the positive control group exceeded that of the untreated control group of the first 6-months of the study period. The skin reaction in the test groups corresponded to the skin reactions to those in the treated control group for the first 6-months of the study. These reactions consisted of reddened spots on the dosage site. Tumors were noted 100% of the positive control groups. No histologic examination of tissues taken from post mortem and sacrificed animals at this time.

Draize Patch Test Study (non-occlusive Patch)

The test was performed on six human subjects. Five-tenths ml of the test solution was placed on a patch which was then applied to the arm of the subject where it remained for 24 hours. This procedure was repeated until ten applications had been made. A ten day rest period was allowed after which a challenge application was made. The test material consisted of bacteriostat CH 3565 0.5% suspension in 1% ivory soap solution.

Results

Bacteriostat CH 3565 is a very mild fatiguing agent, in the concentration test.

Draize Patch Test Study (non-occlusive Patch) - 2.5% Suspension in 5% Ivory Soap Solution

The test was performed on six human subjects. Five-tenths ml of the test solution was placed on a patch which was then applied to the arm of the subject where it remained for 24 hours. The patch was then removed and reactions graded and recorded. Following a rest period of 24 hours a patch was reapplied for 34 hours. This procedure was repeated until ten application had been made. A ten day rest period was allowed after which a challenge application was made. Bacteriostat CH 3565 as a 2.5 suspension and 5% ivory soap solution is a fatiguing agent.

5

001955

Repeated Insult Patch Test 1%, 2.5%, and 10%

Bacteriostat CH 3565 was compared with hexachlorophene in this study. Both CH 3565 and hexachlorophene were applied to the form of either a 10%, 2.5%, or 1% concentration in petrolatum. Test samples were applied to one-inch square swatches of non-woven cloth. The patch was held to the skin under occlusive, impermeable plastic tape. The test patches were applied to the backs of the subjects, five to the right, five to the left of the midline, varying in order so that each material occupied each of the possible positions with approximately equal frequency. The subjects were instructed to remove the patches 23 hours after application. The patches were reapplied to the same area on each of 10 consecutive days unless the severity of the reaction made reapplication inadvisable, in which case that patch was discontinued.

Results

The 10% concentration of both hexachlorophene and CH 3565 were highly irritating; the 2.5% and 1% concentration of hexachlorophene and bacteriostat CH 3565 were comperable.

Repeated Insult Patch Test - 0.25%, 0.5% CH 3565

The preceding test was repeated on 12 human subjects, but this time utilizing 0.25 and 0.5% suspensions by CH 3565 in 1% Ivory Soap solution.

Results

Both the 0.25 and the 0.5% suspension of CH 3565 and hexachlorophene causes moderately severe primary irritation in all subjects.

Sensitization Study

Twenty-five males were used as subjects. The procedure consists of a course of five forty-eight hour exposures with one day intervals between exposures. If the test agent is non-irritating, it is tested at 25% concentration in petrolatum. Each exposure is to exactly the same site, usually an extremity, which has been irritated by a prior twenty-four hour treatment with 5% aqueous solution of sodium

001955

lauryl sulfate. If the test agent is intrinsically irritating, it is used at the threshold concentration for irritancy, and the site is not pretreated with sodium lauryl sulfate. Two weeks after the last exposure, a new skin area is challenged by forty-eight hour patch test, usually 10% in petrolatum. The challenge is applied to a normal area pretreated for one hour with sodium lauryl sulfate; this is the provocative challenge test for the detection of marginal states of sensitization. Readings were made for three successive days. The patch test consists of a 1.5 inch square of non-woven cloth to which about 0.75 grams of test agent is applied. It is held to the skin under an occlusive, impermeable dressing of plastic tape.

Results

No sensitization.

Subacute Dermal Toxicity (Rat- 1 Week)

Six rats (three male, three female) with an average weight of 130 grams were used in this experiment. Before beginning the experiment the hair was removed from the backs of the animals by shaving. 0.4 cc of a 5% suspension of the compound with gum arabic which was prepared fresh daily, was then applied to the diluted area. In order to prevent licking the animals were locked in a narrow cage for three hours after application and subsequently the application area (approximately 1 square inch) was wiped off with a damp sponge. Skin findings were evaluated in each rat 24 hours after - respectively prior to the next application. The compound was applied once daily for one week - always on the same skin areas, except for Saturdays and Sundays.

Results

No local irritant effects were produced in any of the animals. No resorptive toxic effects were noted in any of the animals.

Subacute Dermal Toxicity (Rat - 4 Week)

Ten rats (five male and five female) were used in this study. The average weights

BEST AVAILABLE COPY

7

001955

of the animals at the beginning of the study was 130 grams. The hair was removed from the back of the animals by shaving. 0.4 cc of a freshly prepared 2.5% aqueous suspension of the compound in gum arabic was applied to the denuded area. In order to prevent licking the animals were locked in a narrow cage for three hours after application and subsequently the application area (approximately 10 square cm.) was wiped off with a damp sponge. Evaluation of the skin findings in each case 24 hours after, respectively prior to next application. The application was repeated once daily over a period of four weeks (except Saturdays and Sundays) always on the same skin area.

Results

Daily application of 0.4 cc per animal once daily over a period of four weeks of 2.5% of suspension of a compound with gum arabic produced no local irritation or resorptive toxic effects in any animals.

Subacute Dermal Toxicity (Rabbits)

Five albino rabbits were utilized in this study. The animals were housed individually and food and water were available to the animals ad libitum. The rabbits were divided into three groups of three in such a manner that each group contained at least one male and one female. One group of animals received the control soap and the remaining groups received one of the bacteriostat-containing soaps. The sample labeled control soap was Ivory Soap, the sample labeled 14703 was Ivory Soap containing bacteriostat CH 3565, the sample labeled 14891 was Ivory Soap containing an unstated dighty compound. Each material was applied topically at a dosage level of 100 mg of the sample as received per kg of the body weight per day. Each material was applied once daily for a total of three consecutive applications. Initially and periodically thereafter the fur was removed from the ventral surface of the rabbits using electric clippers. The individual test dose was weighted on a 3 X 3 inch glassine weighing paper and

6

then moistened with sufficient distilled water to form a paste. The paste was spread evenly over the weighing paper and the paper was applied to the previously clipped skin surface. The trunk of the animal was then wrapped with gauze which was secured with adhesive tape. Each daily exposure period was approximately 23-1/2 hours. At the end of each exposure period the binders were removed and the exposed area was thoroughly sponged using a moistened towel. The exposed skin area was examined for gross signs of irritation and the animals were observed for gross signs of systemic toxicity and/or pharmacological effects. The animals were observed daily for skin irritation and systemic effects for a total of seven days. Body weights were recorded initially and at termination of the study. At the end of the observation period the surviving rabbits were sacrificed by intravenous air embolism, and a gross autopsy was performed on each rabbit. From each rabbit the following tissues were preserved in 10% Formalin: skin, lung, heart, liver, gall bladder, kidney, adrenal, spleen, stomach, small intestine, cecum, urinary bladder, gonad, and bone marrow (sternum). Microscopic examinations were made of liver, and kidney. The remaining tissues were held for future references.

Results

Following the initial dermal application the exposed skin area of all animals generally showed mild erythema and spotty areas of blanching. Following the second and third applications the skin showed moderate erythema, mild edema, and blanched areas. Following the final application the edema subsided promptly, and the erythema subsided within an additional three to five days. The majority of the animals showed moderate or marked atonia and mild desquamation during the final days of the observation period. Throughout the ten day period, all rabbits which received the experimental compounds exhibited essentially normal appearance and behavior and gained weight. All samples, including the Control Soap, produced

9

001955

moderate to marked skin irritation under the condition of this test. The observed appeared to be reversible in nature. Gross autopsies and microscopic examination of a section of liver and kidney from each rabbit revealed no pathology which could be associated with dermal application of the experimental compound.

Sensitization (Guinea Pig)

Thirty-three white male guinea pigs were used in this study. The hair on the backs of the animals was removed with a Depilan (composition: calc., thioglycol., calc., hydrate, potassium citrate) several hours prior to test injection. Either 0.05 or 0.1 cc of a suspension of CH 3565 was injected intracutaneous into the test sides of the animals. The animals were injected daily for 10 days. The challenge injection was given one week after the 10th injection. The test sites were evaluated 24 hours after the 11th injection.

Results

No sensitization.

Allergizing (Guinea Pig)

Sixty male albino guinea pigs were used in the test. The guinea pigs were shaved with an electric shaver on the back left of the spine, and on the outside of the left pinna prior to the application, approximately every third day during the period of application, as well as at intervals of one week during the intermediate period.

10

11
001955

Furthermore the hair around the left nipple was sheared off. In analogous fashion hair on the right side of the back, at the right pinna and the right nipple removed prior to the after-treatment.

Concentration of the material applied was 50 mg/L. Once a day, over a period of 21 days - excepting Sundays - an area of 2 cm X 2 cm within the depilated area on the back (always the same spot) was moistened with the test substance with a soft brush. The depilated zones on the left pinna and the left nipple were similarly treated. Forty-three days after the start of the applications, the corresponding areas on the right side of the body were treated once in the manner described. Guinea pigs were weighed upon arrival, and again one day prior to the first application, one day after the last application and one day after the after-treatment.

IR

Irritation on the back was observed throughout the experiment. Reddening was observed on the pinna. Slight reddening was observed on the nipple. Weight increase normal.

In the cases CH 3565 at the concentration study did cause neither primary irritation or allergizing effects.

Toxicity

The most irritating concentration of each agent in petrolatum was applied to the back under occlusion in ten white human subjects for 24 hours. Immediately following removal of the patches each site was exposed to FS-20 ultraviolet light for 10 inches for one and one half times the minimal erythema dose. A collateral test was prepared in the same manner, but using petrolatum as a control. Both test and control, were rated one to four as a erythema 24 hours following the ultraviolet exposure.

BEST AVAILABLE COPY

12
001955

results

o significant photo toxicity.

photosensitization (Human)

ix areas (1/2" diameter) were designated on each arm of each subject. Bacteriostat CH 3565 was prepared in concentrations from 1.25% to 2%. A drop of each solution was placed on its designated area and allowed to dry. The areas were then exposed to a cold quartz lamp for 20 seconds duration at a distance of 12 inches. After radiation the sites were left uncovered. This procedure was repeated every 24 hours, five days a week, for a total of ten treatments unless reactions intervened.

results

irty-nine subjects out of forty-five developed a severe reaction in areas containing any amount of TCSA. None developed any reaction in the areas containing Bacteriostat CH 3565 alone.

Bacteriostat CH 3565 had no primary photosensitizing nor cross-sensitization capabilities when tested under the conditions described.

Absorption through intact skin (human)

After six hours of contact with the forearm, 2% solution of CH 3565 in 10% Ivory, 5% Dial base, and 15% Colgate base soap, were quantitatively collected and analyzed for CH 3565 by UV spectrophotometric measurements.

results

Computed averages indicate recoveries of at least 95% CH 3565 from each formulation.

4

001955¹³

Eye Irritation (FHSA)

The procedure followed for the eye irritation test was in accordance with the evaluation of eye irritation in Sections 191.11 (g) (3) and 191.12 of the Federal Regulations. These regulations require examination for irritation at 24, 48, and 72-hour intervals after ocular instillation.

Eye irritation was scored quantitatively using the method of Draize et al which is consistent with the descriptive criteria for evaluation of eye irritation in the Federal Regulations.

The Resulting Eye Irritation Scores Are as Follows:

Twenty-four hours = 92.0; 48 hours = 86.3; 72 hour = 82.0. Severe irritation scores were recorded of the cornea at each examination period.

According to the results of this test, the test material would be classified as an eye irritant.

Acute Eye Irritation (Draize)

Two groups each consisting of three albino rabbits were used to evaluate the eye irritating properties of the test material.

The test method employed was patterned after that of Draize et al. Exactly 100 mg of undiluted test material was instilled into the conjunctival sac of the right eye of each rabbit in both test groups. In the first group the test eyes were then rinsed with 20 ml of tap water two seconds after instillation of the test material. The test eyes of the rabbits in the second group were treated in the same manner after a four-second contact period. The left eye of the animals served as a scoring control.

One, 24, 48, 72, 96 hours, and 7 days following the initial instillations, the cornea, iris, and palpebral conjunctiva were examined individually and graded for irritation and injury according to the standard scoring system. A score of 110 points indicates maximal irritation and damage to all three ocular tissues. A score of

13

Zero indicates no irritation.

A descriptive rating assigned was one of the following, each of which characterizes a particular level of ophthalmic irritation and damage:

Non-Irritating
Practically non-Irritating
Minimally Irritating
Mildly Irritating
Moderately Irritating
Severely Irritating
Extremely Irritating
Maximally Irritating

Results

The mean irritation scores (washed eyes - two-second contact) were as follows:

One hour - 38.7; 24 hours = 59.3; 48 hours = 56.0; 96 hours = 31.9; 7 days = 24.4.

These scores indicate that bacteriostat is a severely irritating substance to the eyes.

The mean irritation scores (washed eyes - 4-second contact) were as follows:

one hour = 42.0; 24 hours = 36.6; 48 hours = 20.7; 72 hours = 10.7; 96 hours = 5.3; 7 days = 0.1.

These scores indicate that bacteriostat under these conditions is a moderately irritating substance.

Acute Dermal Toxicity (Rabbits)

Young adult rabbits with an average weight of 2.5 kilograms were employed as test animals. Twenty-four hours prior to the dermal applications, the backs of the rabbits were shaved free of hair with electric clippers. The shaved area on each animal constituted about ten per cent of the total body surface area.

On the testing day, the rabbits received skin applications of the test material in the form of a slurry in propylene glycol at several selected dose levels. Groups for each dose level consisted of four rabbits (two male and two female). After each application, the exposure site was covered by wrapping the trunk of the animal with an impervious plastic sheeting which was securely taped in place. This

14

001955

plastic wrap insured intimate contact of epidermis and test material.

The test material remained in contact with the skin for 24 hours. Any reactions displayed by the animals were observed and recorded during the contact period, after which the plastic sheeting was taken off each test rabbit and all residual test material removed. The exposure sites were examined for local skin reactions and the animals returned to their cages. Observations for mortality, local skin reactions, and behavioral abnormalities were continued for a total of 14 days following the skin applications. Initial and final body weights were also recorded. The dosage levels employed were 4.6 g/Kg, 10.2 g/Kg.

Results

No untoward behavioral reactions were noted among any of the animals tested.

Local skin reactions characterized by moderate erythema and edema were noted at the end of the 24-hour contact period. By the seven-day point of the observation period the skin at the application site was very dry and/or necrotic. No change was noted at the end of the 14-day observation period. Hair regrowth was completely inhibited. Necropsy of animals which died during the study as well as of those sacrificed at the end of the 14-day observation period did not reveal any significant gross pathologic alterations in the tissues and organs examined except for the dermal alterations noted above.

No deaths occurred at the 4.6, and at 6.8 g/Kg dosage levels. At the 10.2 g/Kg dosage levels three out of four animals succumbed. One of these animals died at the third day of observation. The remaining died on the fifth day. Acute dermal LD₅₀ was calculated to be greater than or equal to 9.3 g/Kg.

15