MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

CASWELL FILE 311

1/8/67

PC

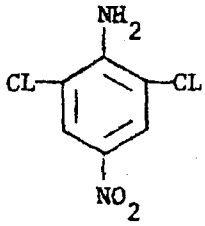
031301

Trade Name : Botran

Chemical Name: 2:6-dichloro-4-nitroaniline

Empirical formula : $C_6 H_4 N_2 O_2 Cl_2$

Structure :



Molecular wt : 207.03

Physical state : Solid

M.P. : 192-194°C

Color : Yellow

Solubility : Insoluble in water - poor solubility in most organic solvents

Co. : The Upjohn Company

Stability : Stable to hydrolysis - is reduced to the corresponding phenylenediamine by zinc and acid.

Use : Control of Botrytis (Grey Mold) in lettuce

Impurity [redacted]

(1)

Botran #1

Acute Rat Oral	:	LD_{50} = 4040 mg/kg
Acute Rat Oral (4% Formulation)	:	LD_{50} = >8000 mg/kg
Acute Rat Oral (8% Formulation)	:	LD_{50} = >8000 mg/kg
Acute Rat Oral (50% Formulation)	:	LD_{50} = >8000 mg/kg
Acute Mouse Oral	:	LD_{50} = 1500-2500 mg/kg
Acute Cat Oral	:	LD_{50} = >500 mg/kg
Acute Guinea Pig Oral	:	LD_{50} = 1450 mg/kg
Acute Rat I.P.	:	LD_{50} = 1460 mg/kg
Acute Mouse I.P.	:	LD_{50} = 2500 mg/kg
Acute Mouse I.P. (8% Formulation)	:	LD_{50} = 2385 mg/kg
Acute Rat Subcutaneous	:	LD_{50} = >5000 mg/kg
Acute Mouse Subcutaneous	:	LD_{50} = >6000 mg/kg
Acute Mouse Dermal	:	LD_{50} = >5000 mg/kg
Acute Rat Inhalation (7 hrs)	:	No adverse effect at a 0.381 mg/~~kg~~ l conc
Acute Rat Inhalation (75% Formulation) (1hr)	:	No adverse effect at a 21.6 mg/~~kg~~ conc
Acute Guinea Pig Inhalation (7 hrs)	:	No adverse effect at a 0.381 mg/~~kg~~ l conc
Acute Rabbit Inhalation (7 hrs)	:	No adverse effect at a 0.381 mg/~~kg~~ l conc
Acute Rabbit Dermal (75% Formulation	:	Slight irritant
Rabbit Eye Irritation (Tech)	:	Slight irritant
Rabbit Eye Irritation(8% Formulation)	:	Non-irritant
Rabbit Eye Irritation (75% Formulation)(5 day)	:	Non to slight irritant

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Subacute Rabbit Dermal Irritation	:	Non-irritant
Subacute Rabbit Dermal Irritation (8% Formulation)	:	Non-irritant
Subacute Rabbit Dermal Irritation (75% Formulation)(21 day)	:	Slight irritant
Subacute Rabbit Dermal Irritation (75% Formulation)(5 day)	:	Non to slight irritant
Guinea Pig Dermal Sensitization	:	Not a sensitizer
Subacute Rat Oral (28 dyas)	:	Liver enlargement
Subacute Rat Oral (28 days)	:	Liver enlargement is reversible
Subacute Cat Oral	:	Effect level $\cong$ 200 mg/kg
Chronic Rat Oral (6 months)	:	No effect level $\cong$ 300 ppm (22-25 mg/kg)

Chronic Dog Feeding (2 year)	:	One year results indicate hemotoxic properties
Chronic Rat Feeding (2 year)	:	One year results indicate possible liver effects. Yellow stain on coat and in urine
Synergism Mouse I.P. Study	:	Acts as a synergist for certain chemicals
Effect on Blood and Blood-forming tissues (Rats)	:	Caused growth inhibition and marked lymphopenia - no adverse effect on red cells
Methemoglobin in Cats	:	Methemoglobin levels were normal
Human Manufactoring Experience	:	No adverse effect

Review on Human Clinical Study: 1/8/67

Compound : Botran (U-2069)

Use = Fungicide

Structure :

Subacute Human Oral Study

Design :

90 day oral study conducted on 30 adult males in double blind basis. One group received one 10 mg tablet of test material daily for 90 days, while the control group received a placebo tablet daily during the 90 day duration. The dosage of 10 mg/day per adult was selected to correspond to an amount of test material somewheat greater than the expected residue on ingested fruits and vegetables, if treated fruit and vegetables were eaten daily in a reported per capita amount. The amount of 10 mg/day per person corresponds to 0.143 mg/kg/day in a 70 - kg human. Total ingested test material for the study is 900 mg.

Clinical symptomology was recorded daily following interrogation of all subjects, and urine, blood and liver function studies were carried out monthly.

Those test done were:

1. urinalysis, including microanalysis
2. hemogram and differential
3. BUN
4. Alkaline Phosphatase
5. Bromsulphalein Test
6. Serum Glutamic oxalo acetec transaminase

Results - Clinical Symptoms:

Subject #20 taking the test material had persistent diarrhea for approximately four weeks during the study, beginning on the ninth day. It cleared spontaneously on the 39th day. Subject #6, taking the placebo had symptoms of persistent sleepiness.

Several subjects had symptoms of nausea for 1-2 days time early in the study. Three of four of these patients were taking the placebo.

Lab Results

Subjects #9 and #25 showed minor BSP changes suggesting possible hepatic insufficiency. Subject #25 received the test material in quantity of 10 mg daily, whereas Subject #9 received the placebo.

The remainder of the laboratory findings were normal.

Conclusions:

No specific toxicologic findings are apparent from the 90 day oral human study. Only one subject, #25 showed any evidence of altered hepatic studies and this change was very minimal, but, however, consistent

BOTRAN

Acute Rat Oral

Ten animals were used per dosage level (individual dosage levels not specified).

Results

Oral LD_{50} equals 4040 mg/kg. Toxic symptoms included prostration within 24 hours, signs of peripheral vasodilatation and gastric damage.

Acute Mouse Oral

LD_{50} equals 1500-2500 mg/kg. This variation probably reflects eratic absorbtion from the GI tract.

Acute Guinea Pig Oral

LD_{50} equals 1450 mg/kg.

Acute Rat Intraperitoneal

LD_{50} equals 1460 mg/kg. Deaths occur within 48 hours after treatment. The compound became aggregated in the peritoneum with the formation of small granuloma and cellular adhesions, mainly on the liver.

Acute Mouse Intraperitoneal

LD_{50} equals 2500 mg/kg. Toxic symptoms were identical with those found in the acute rate intraperitoneal study.

Acute Rat Subcutaneous

LD_{50} equals >5000 mg/kg. The effects of subcutaneous injection were

confined to locate damage, in which the compound became enclosed in a fibrous sac sometimes followed by abcess formation and eventual ulceration through the skin.

Acute Mouse Subcutaneous

LD_{50} equals >6000 mg/kg. The toxic symptoms noted in this study were identical to the symptoms found in the acute rat subcutaneous study.

Acute Mouse Dermal

Ten mice were tested per dosage level of 3.2 and 5.0 mg/kg. Half the animals in each group were tested with abraded skin. The test material was applied as a paste in 10% gum acacia for an exposure period of 24 hours.

Results

None of the animals died or showed any ill effects.

Rabbit Eye Study

2-3 mgs. of the test material was placed in the eyes of four rabbits every day for four successive days.

Results

Apart from a slight inflammatory reaction of the cornea in some of the animals, which was of short duration, there were no signs of irritation.

Subacute Rabbit Skin Irritation Study

Four rabbits were tested with 10 mg of the solid material and 0.1 ML's

of 10% suspension and 3% aqueous solution. Exposure time was six hours a day for five days. These applications were made to both abraded and unabraded skin.

Results

Neither the solid nor the suspension exhibited an irritating effect.

Guinea Pig Sensitization Study

Five guinea pigs were injected intradermally with 0.05 ML's of the test material at two or three day intervals until a total of 10 injections had been made. Two weeks after the final dose a 0.05 ML challenge dose was given. A positive control group was given p-phenylene diamine (a known sensitizer) at the same concentration.

Results

The conclusion drawn is that the test material produced neither priamry irritation nor skin sensitization.

Subacute Rat Oral

Five male and five female rats were tested at the dosage levels of 350 and 140 mg/kg. Treatment was given six days per week times four weeks. Sections of the heart, lung, liver, kidneys, spleen, suprarenal, pancreas, stomach, ileum, ovary or testes, thyroid and brain were taken for histopathological examination.

Results

Average body weight gain of the 350 mg level males showed a 40% inhibition loss of weight as compared to the control animals. The growth of the female animals for this level appeared normal.

9

The livers were enlarged to approximately 155% of the controls by weight. Histologically the liver cells were vacuolated starting usually at the periphery of the lobules and extending to a variable distance into the lobule. Histologically the kidneys appear normal. Hemological studies were also normal.

Animals of the 140 mg/kg level showed liver enlargement with vacuolation of the liver cells. No other effects were noted.

Subacute Rat Oral (twenty-eight days) Study No. 2

Ten newly weaned male and ten females were tested per dosage level of 35, 140, and 350 mg/kg. Treatment was given six days a week for four weeks. After four weeks, half of the animals were sacrificed and the other half maintained on control diet.

Results

The animals receiving 350 mg/kg showed a 33% reduction in body weight gain as compared to the control animals.

The males of the 140 mg/kg level showed a slight reduction in body weight gain. The animals on the 35 mg/kg level showed no loss of body weight gain.

At 28 days the liver weights of the groups treated with doses of 140 and 350 mg/kg were abnormally high. The lowest level (35 mg/kg) had liver weights comparable to those of the controls. After two weeks on control diet the liver weights of the treated animals of the 140 and 350 mg/kg had returned to normal with the exception of the males. These males did exhibit a marked reduction in liver weight but it was not

sufficient enough to be considered normal.

At 28 days the liver cells showed increased vacuolation and swelling. After two weeks on control diet the remaining animals showed normal liver observations.

The conclusion drawn is that liver hypertrophy and vacuolation is reversible.

Chronic Rat Oral (twenty-seven weeks)

A technical grade of the test material was tested at the dosage levels of 3000, 300, and 30 ppm. Ten male and ten female newly weaned rats were used on the highest concentration, 15 males and 15 females were used on the other two concentrations.

The 3000 ppm level is equal to an average daily dose of 230 mg/kg for the males and 270 mg/kg for the females. The 300 ppm is equal to an average daily dose of 22 mg/kg for males and 25 mg/kg for females. The 30 ppm level is equal to an average daily dose of 2.2 mg/kg for males and 2.5 mg/kg for the females.

A pure sample of the test material was also used in this study at a concentration of 3000 ppm. Ten males and 10 females were used for this dosage level. This dosage level is equivalent to an average daily dose of 230 mg/kg for males and 260 mg/kg for females.

At termination of the study sections of the heart, lungs, liver, kidneys, spleen, suprarenal, pancreas, stomach, ileum, bladder, testes or ovary, brain and femoral bone marrow were taken for histopathology.

Results

Deaths were noted in all treatment and control groups. The dosage levels

utilizing the technical material from 30-3000 ppm gave 27, 10, and 20% deaths respectively. The pure material (3000 ppm) gave 15% deaths. The negative controls gave 12%. These deaths tend to indicate that there is another contributing factor besides the administration of the test material. Although these deaths do not completely invalidate the report they certainly cast a shadow over the results.

3000 ppm results - the growth rate of the males and females was reduced to about 80% and 70% of the controls respectively. Livers were enlarged approximately 40% over that of the corresponding controls. The sections of the liver appear to be histologically normal.

Kidneys were slightly enlarged but histologically normal.

Spleen weights appeared to be normal in the male animals and slightly enlarged (30%) in the female animals.

300 ppm test results - the food consumption, growth rate, liver, kidneys, and spleen values appear normal. No histopathology was noted in these organs.

30 ppm test results - no effects whatsoever were detected.

3000 ppm test results using the pure material - male growth rate was slightly reduced (10%); normal in females. Livers showed a slight enlargement, but were histologically normal.

Summary

The results from the aforementioned test indicate little or no toxic effects at 30 or 300 ppm. At 3000 ppm the test material appears to cause moderate reduction in growth and hypertrophy of the liver.

Acute Cat Oral

Two cats received a single dose of 500 mg/kg by mouth.

Results

There was no effect on one cat. The second cat showed ataxia 24 hours later and was restored to normal after an additional 48 hours.

Subacute Cat Oral

Two cats received daily doses of 200 mg/kg for a total of seven doses.

Results

One cat showed no ill effects. The other was slightly ataxic several hours after the third dose. No other effects were noted.
Postmortem examination three days post treatment revealed no gross or histological pathology which could be related to treatment.
No evidence of the formation of methemoglobin was noticed in these cats.

Effects on Blood and Blood Forming Tissues

Five male and five female newly weaned rats were tested at the dosage level of 400 mg/kg. Exposure period was five days a week for four weeks. Two other groups of five male and five female newly weaned rats received a dosage of 200 and 400 mg/kg of 4-nitroaniline (known to be hemotoxic).

Results

The test animals receiving the 400 mg/kg dose showed a 50% reduction in body weight gain as compared to the control animals. No Heinz bodies

were detected and reticulocyte counts were normal. Bone marrow possibly slightly less active than normal.

The male test animals receiving 400 mg/kg of 4-nitroaniline showed a 25% reduction in body weight gain, females were normal.

Heinz bodies were identified in the blood of one of the three males and in none of the females. Marked reticulocytosis was noted in both sex. The reticulocyte count in males was approximately 11 times greater than the control values. This same count in the females was approximately seven times greater than the control value.

Bone marrow was possibly slightly more active than normal.

Males receiving the 200 mg/kg of the 4-nitroaniline showed a 30% reduction in body weight gain. Females were normal.

Heinz bodies were identified in the blood of all three males and in one of the three females. Particular site counts increased in both sex; four times the control value in males and twice the control value in females.

Bone marrow possibly slightly more active than normal.

Study No. 2

A group of six newly weaned male rats received a dosage of 400 mg/kg twice daily, five days a week for two weeks. Two other groups of six newly weaned male rats received a dosage of 200 and 400 mg/kg of 4-nitroaniline twice daily, five days a week times two weeks.

Results

The test material at 400 mg/kg produced a small loss in weight over the test period. Red blood count, cell hemoglobin content and packed cell volume of blood were normal. Lymphocyte count was reduced to 65% of normal. Spleen appeared normal.

The 4-nitroaniline at 400 mg/kg produced one death during the second week. Red cell count was reduced approximately 45% of control. Many of the red cells showed polychromasia and others were nucleated. Blood hemoglobin was reduced to 70% of the corresponding control. Lymphocyte count approximately doubled. Spleen grossly enlarged (average 300% of controls by weight).

4-nitroaniline at 200 mg/kg showed reduced growth (60% of controls). Red cell count reduced (65% of control); some of the red cells showed polychromasia and others were nucleated. Blood hemoglobin and packed cell volume appeared normal. Spleen enlarged (average 200% of control by weight).

The test material completely inhibited growth and also caused a marked lymphopenia, but had no effect on the red cells. In contrast, 4-nitroaniline at the same or half the dosage caused severe hemotoxic effects as shown by reduction in red cell counts and greatly increased rediculocytes and presence of heinz bodies.

Methemoglobin Formation in the Cat

Three cats were given the test material at a dosage level of 500 mg/kg by mouth and three other cats were given 4-nitroaniline at a dosage level of 100 mg/kg by mouth. (I assume this was a single dose treatement). The portion of methemoglobin in the blood was measured spectrophotometrically.

Results

Methemoglobin levels using test material vary from 0 to 10% and are

considered of little significance. The cats receiving 4-nitroaniline showed methemoglobin levels from 28 to 56%, which is considered significant.

Summary

The results of this experiment indicate that the test material at a dose of 500 mg/kg has no methemoglobin-forming properties. In contrast, 4-nitroaniline in 1/5 of the dose converted more than 50% of the blood hemoglobin to methemoglobin.

Acute Rat Oral (8% W/W Dust)

The formula used in this study contained 8% Botran, [redacted] and [redacted].

Five rats were tested per level of 8000, 5000, 3200, and 2000 mg/kg.

Results

No deaths occurred. LD_{50} equals >8000 mg/kg.

Acute Mouse I. P. (8% Formulation)

Ten mice were tested per level of 4000, 2500, 1600, 1000, and 630 mg/kg.

Results

LD_{50} equals 2385 mg/kg (a range of 2046-2781). Excessive yellow urine and depression was noted at the 1600 mg/kg level.

Rabbit Skin Irritation Study (8% Formulation)

Liberal amounts of the 8% dust was applied to the abraded and unabraded skin of two rabbits once a day for five days.

Results

No sign of skin irritation was detected grossly.

Rabbit Eye Irritation Study (8% Formulation)

A small amount of the test material was placed in the left eye of two rabbits twice daily for five days.

Results

The oculoirritant property of the test material was nil.

Human Manufacturing Experience

The Yorkshire Dyeware Chemical Company has manufactured and used botran in the preparation of dyes for the past ten years. During this time no special precautions such as the wearing of eye shields, face masks, and the use of draughthoods were used. No ill effects from such manual operations as drying, weighing for packaging has been reported to them. They indicate that the exact exposure is impossible to determine because this product is used intermittently in their organization. They indicate that any one of several individuals may have had two or three years of intermittent contact. They also indicate that since the dry powder is a dusty one, small quantities may have been inhaled or ingested. Based on this information we must conclude that there have been no industrial poisoning cases with this product in the Yorkshire Dyeware Chemical Co.

Chronic Dog Feeding (13 week and 52 week reports)

The test material used in this study was 2,6-Dichloro-4-nitroaniline with 0.09% p-nitroaniline.

Four male and four female pure bred beagles were tested per dosage level of 20, 100, and 3000 ppm. (These dosage levels are equivalent to approximately 0.6, 3.0, and 90 mg/kg per day. (Portions of 31 tissues were examined from one male and one female from each group which were sacrificed at 14 weeks.

Results

Two of four high level males showed a significant body weight loss, 28 and 18%. One female at the high level showed a slight weight loss. The remaining dogs of the high level and lower levels with the exception of one female receiving 100 ppm, all maintained or gained weight. One male dog of the 3000 ppm level was slightly emaciated and from the eighth week of the study appeared slightly icteric. This animal also showed weeping ezzematous-type lesions on the flank and neck region. One female receiving 100 ppm also showed a hair loss around the ears and neck and anal region.

Hemological examination - dogs of the 20 and 100 ppm were comparable to the control dogs. The dogs receiving 3000 ppm showed evidence of hemolytic anemia. All males and two females at this level showed a decrease in hemoglobin and packed cell volume at 13 weeks. Three of four males also showed leucocytosis. Changes in the erythrocytic series were evident at 8 and 13 weeks due to the findings of pale and microcytic cells. Poikilocytosis and anisocytosis and nucleated red cells were also

slightly higher for the animals receiving 3000 ppm.

Urinalysis: Collected samples of the 3000 ppm level indicated a more highly pigmented urine and more casts than are found in the control urine.

Biochemical Results

No. 1

Serum alkaline phosphatase was elevated at 11 and 13 weeks for the 3000 ppm dogs.

No. 2

Serum glutamic pyruvic transaminase was elevated at 8, 11, and 13 weeks for the 3000 ppm animals.

No. 3

Serum glutamic oxalacetic transaminase was elevated at 8 weeks for the 3000 ppm level and return to normal at 13 weeks.

No. 4

Prothrombin time was increased at 8 weeks in the 3000 ppm level.

No. 5

Serum protein showed a slight reduction in these animals. BUN, methemoglobin, fasting blood sugar, and creatinine was within normal values for all dogs.

At gross autopsy, the organ to body weight ration showed slightly enlarged livers and kidneys at the 3000 ppm level.

Histological examination conducted at 14 weeks revealed moderate degenerative and chronic inflammatory hepatic changes in the 3000 ppm animals. Similar changes but at a moderate degree were also seen at

the 100 ppm level. This report states that changes of the magnitude seen at the 100 ppm have been seen from time to time in other control animals.

Results at 52 Weeks

3000 ppm - All animals show a thrombocyte increase; females show drop in hemoglobin.

100 ppm - All animals show a slight thrombocyte increase. One male shows leukocytosis. One male shows hemolytic anemia.

20 ppm - No change.

Chemistry

3000 ppm - Animals show an increase in SGOT and SGPT and slight elevation in prothrombin time; also a decrease in serum protein. Also a slight BSP retention was evident.

Chronic Rat Feeding Study (2 Year)

35 male and 35 female rats were tested at the dosage levels of 10, 50 and 1,500 ppm from week one through week four (equivalent to approximately 1.6, 8.3, and 249 mg/kg per day). From week five on the dosage levels per group were doubled. At 13 weeks five males and five females from each group were sacrificed. Gross and histological observations were made.

13 week Results

The weight gain and food consumption of the 3000 ppm (1500 ppm level) level were suppressed. The 20 and 100 ppm levels were comparable to the control group.

Hemograms revealed nothing of consequence at any level. The 3000 ppm level animals showed an increase in the liver and kidney organ to body weight ratio.

Histological examination - Animals of the 3000 ppm level showed mild hepatic cell changes and slight adrenal cortical atrophy in three of ten animals examined.

52 Week Results

3000 ppm - General increase in irritability; yellowed coat seen from 48 weeks; yellow stain in urin seen from 52 weeks. Slight depression of body weight gain. Plasma yellow in appearance.

100 ppm level - One male death at 14 weeks. General increased irritability seen from 24 weeks; slightly yellowed coat seen from 48 weeks; yellow stain in urine seen from 52 weeks. Other aspects appear normal.

20 ppm level - All aspects appear normal.

Control Group - Three deaths noted.

Acute Rat Inhalation

Ten rats were exposed to an aerosol of the 8% test material dust for a period of seven hours at a concentration of 0.381 MG/L.

Results

Throughout the exposure period there were no mortalities, signs of toxicity, nor pharmacological effects. At autopsy some redness of the lungs was noted in several rats. Other organs appeared normal.

Acute Guinea Pig Inhalation

Seven guinea pigs were exposed to an aerosol of the 8% test material (dust) for a period of seven hours at a concentration of 0.381 MG/L.

Results

Throughout the seven hours of exposure there were no mortalities, signs of toxicity, nor pharmacological effects. At autopsy some redness of several lungs was noted. Other organs appeared normal.

Acute Rabbit Inhalation

Two rabbits were exposed to an aerosol of the 8% test material (dust) for a period of seven hours at a concentration of 0.381 MG/L.

Results

During the seven hours of exposure there were no mortalities, signs of toxicity, nor pharmacological effects. At autopsy all organs appeared normal.

Rabbit Eye Irritaion Study (75% Formulation) (Five Days)

A small amount of the test material was instilled into the right eye of two rabbits twice daily for five days. Eyes were examined daily.

Results

The oculoirritant property of the test material was very slight.

Subacute Rabbit Dermal Irritation Study (75% Formulation) (Five Days)

Liberal amounts of the wettable powder were applied to the abraded and

unabraded skin of two rabbits. Treatment was once daily for five days.

Results

The material was nontoxic or possibly sightly irritating to the abraded skin.

Synergism IP Mouse Study

Botran was administered to mice intraperitoneally in combination with other selected drugs. Ten animals per dosage level were utilized. The other drugs include librium, hydralazine hydrochloride, aspirin, provest, medrol, panalba (tetracycline phosphate complex and novobiacin sodium), guanethidine.

Results

Botran was the synergistic co-respondent in five of the seven studies conducted. The two drugs in which synergism was not present ar guanethidine and panalba.

Acute Rat Inhalation (One Hour)

Five male and five female rats were exposed for one hour to a dust of Botran 75% wettable powder at a concentration of 21.6 MG/L. Experimental animals were maintained for 14 days after exposure. Fourteen days after the exposure the animals were sacrificed and examined for gross changes and the lungs, heart, liver, kidney, and brain were weighed.

Results

No response to exposure was noted.

Acute Rabbit Dermal (75% Formulation)

Two male and two female with abraded or unabraded skin were tested per dosage level of 632, 2000, and 6320 mg/kg. Exposure time was 24 hours. Animals were observed for 14 days.

Results

One rabbit of the low level (632 mg/kg) died 12 days after dosage. Death was attributed to acute enteritis. The other rabbits remained in good physical condition with no signs of systemic toxicity being noted. Skin irritation consisted entirely of some erythema, ranging from slight to moderate. Animals appeared normal by day eight.

Summary

From the aforementioned data it can be concluded that 75% Botran formulations are mildly irritating to the skin of rabbits.

Subacute Rabbit Dermal (75% Formulation) (Three Weeks)

Twenty male and twenty female (half abraded and half unabraded) were tested per dosage level of 500 and 1500 mg/kg. Duration of treatment was six to eight hours per day for five days per week for three weeks. At 21 days three males and three females from each group were sacrificed with the remaining animals being maintained until day 35.

Results

One rabbit of the 1500 mg/kg level with abraded skin died on day 34 apparently of pneumonia. The remaining rabbits appeared to be in good condition throughout the study, with only slight erythema being evident at the end of the days exposure. This condition generally disappeared

over night. No signs of systemic toxicity were noted.

Summary

These results indicate that the 75% formulation of Botran is only slightly irritating to the skin of rabbits. For other determinations made and considered normal were higher weight gains, hemalogical values, BUN, methemoglobin, serum glutamic-oxalacetic transaminase, serum glutamic pyrubic transaminase, absolute organ weights, histopathological observations at 21 and 35 days.

Rabbit Breeding and Teratogenic Study (One Generation)

11-14 female rabbits were tested per dosage level of 0, 100, and 1000 ppm. The control and treated rabbits were maintained on control diet (without test material) until the eighth day of pregnancy. The treated groups were then given the compound at the predetermined dosage level until the sixteenth day of pregnancy.

Pregnant females were allowed to deliver naturally while nonpregnant animals were sacrificed for examination of the uterus. The parent female animals and the pups were sacrificed on the twenty-first day of weaning.

The parent females were observed for fertility, fetal resorption, abortion, delivery, length of gestation period, and lactation performance.

Results

Two females of the 1000 ppm level died due to pneumonia and mastitis.

One control rabbit died during the study due to enterities and diarrhea.

Breeding Cycle

No adverse effects were observed relative to the fertility, fetal resorption, abortion or delivery. Observations of the treated groups with respect to live births, size of litter, sex ratio, viability of the new-born, 24 hour and 21 day survival were comparatble to the control animals.

Parental Pathology

No gross pathologic lesions attributable to the test material were observed in any parent doe. In counting implantation sites in the uterus no marked discreptance between the number of sites found and the number of pups could be establised. Three fetal resorptions were noted in a controlled doe and one resorption was noted in a 1000 ppm doe.

Offspring Pathology

There were no gross morphologic abnormalities in the offspring of the control or treated groups. There were a small number of abnormalities of the sternebrae and 13th rib. These findings were not compound or dose related. They are considered to be spontaneous and unrelated to treatment.