2/28/95

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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

FEB 28 1995

MEMORANDUM

SUBJECT: Gibberellic Acid Registration: A List D Chemical (No. 043801); Case No. 4110. Review of Toxicological Data Package for Abbott's 4% mixture of Gibberellins GA_4A_7 (ProCone® (ABG-3149)) End-Use Product. Submission No. 8463857; MRID Nos. 431199-04,-05,-06, 430451-01,-02, DP Barcode D202413 and D210629

FROM: Freshteh Toghrol, Ph.D., Chemist F. Toghrol
Biopesticide & Pollution Prevention Division

THRU: Roy Sjoblad, Ph.D., BPPD Senior Scientist R Sjoblad
Biopesticide & Pollution Prevention Division

TO: Phillip Hutton, Team Leader
Biopesticide & Pollution Prevention Division

INERT INGREDIENT INFORMATION IS NOT INCLUDED

ACTION

Abbott requests registration of an end-use product, ProCone® to be used on pine nuts. The end-use product ProCone® contains 4 % Gibberellins GA_4A_7 as its active ingredient and [redacted] which is exempt from tolerance requirements when used as an inert. To support this registration, Abbott has submitted a proposed label and a Confidential Statement of Formula (CSF; dated 12/28/93) for a basic formulation of the unregistered end-use product, ProCone (ABG-3149). Abbott has also submitted the following toxicological studies for this end-use product: an acute oral toxicity study in rats, an acute dermal toxicity study in rabbits, an acute inhalation toxicity study in rats, a primary eye irritation study in rabbits, and a primary dermal irritation study in albino rabbits. No dermal sensitization data (GLN No. 152-15) was provided with this package. BPPD has been asked to confirm that no dermal sensitivity studies are required (Denise Greenwood, Barcode D210629, dated 1/5/95).

BPPD CONCLUSIONS

1. BPPD has no objection to registration of ProCone® to be used in or on Pinacea family Conifer species. BPPD concludes that the use of ProCone on pine nuts is a food-use but is exempt from tolerance requirements, because the toxicology data base is complete and would support a tolerance exemption (see also conclusion 4).

2. Based on toxicological data submitted by the registrant, BPPD has no objection to the registration of ProCone (ABG-3149) because addition of an inert ingredient (96%) has not increased the toxicity of the active ingredient.

3. BPPD waives the data requirement for a dermal sensitization study (GLN No. 152-15) for this end-use product. However, any observed incidents must be reported.

4. BPPD recommends that the existing tolerances for gibberellic acid (A_3, A_4 A_7) found in 40 CFR §180.224 (a)(b) should be revoked. The exemption from tolerance found in 40 CFR §180.1098 should be revised for all Gibberellins as was suggested by J. T. McClintock in a memo dated 8/19/93. BPPD recommends that the revised exemption from tolerance found in 40 CFR §180.1098 could read as follows:

§180.1098 Gibberellins (A_3, A_4, A_7); exemption from the requirement of a tolerance.
Gibberellins are exempted from the requirement of a tolerance when used as a plant growth regulator at application rates less than 20 grams of active ingredient per acre (20 g ai/A) in or on all raw agricultural commodities.

ProCone® Proposed Label

According to the proposed label, ProCone will be used as a plant growth regulator to promote pollen and seed cone production of Pinacea family Conifer species, such as Larix leptolepsis (Japanese larch), Picea engelmannii (Engelmann spruce), Pinus thunbergii (Japanese black pine), and Tsuga heterophylla (Western Hemlock). The proposed label suggests three kinds of applications: injection, foliar application, and topical application to single buds. The three applications and the application rates are given below:

a) An injection into main stems of trees, 5 to 20 centimeters in diameter, of 0.75 to 1.5 mg active ingredient in 0.018 to 0.036 ml of undiluted ProCone/cm^2 cross sectional area at breast height. Doses per stem diameter at 10 cm, 15 cm, and 20 cm are 58.9-117.8 mg, 132.5-265 mg, and 253.5-471.0 mg per tree respectively.

b) A foliar application to trees 0.5 to 5 meters in height in orchards or containers. The entire upper crown should be wetted to run-off with a spray solution containing 200-400 mg Gibberellins A_4,A_7/liter.

c) A topical application to single buds. Apply 210 µg Gibberellins A_4,A_7

2

to each bud in 0.1 ml of 50% v/v ProCone in water. ProCone can also be diluted with 95% ethanol.

STUDY SUMMARIES

152-10 An Acute Oral Toxicity Study in Rats (MRID 431199-04)

The data submitted by the registrant supports the conclusion that the acute oral LD_{50} of Procone® (ABG-3149) was 2100 mg ABG-3149/kg with 95% confidence limits of 1510 to 2910 mg ABG-3149/kg. The material was administered at dosages of 500, 2500, 2550, and 5000 mg ABG-3149/kg to groups of five male and five female Sprague Dawley rats. The animals were observed for signs at 1, 2½ , 4 hours following administrations on the day of dosing and daily thereafter for 14 days. The mortality rate was 0% for 500 mg ABG-3149/kg. Clinical signs of toxicity included rales decreased feces in 500 mg/kg dosage group on days 1 and 2 post dosing; and lacrimation, ataxia, decreased feces, decreased activity and prostration in rats of both sexes at 2500,3550, and 5000 mg/kg. The mortality rates for 2500, 3550, and 5000 mg ABG-3149/kg, were 50% (one male and 4 females), 60% (5 males and one female), 100% (5 males and 5 females), respectively. Gross patologyical observations during necopsy in rats that died during the study included mottled stomach, intestines, distended bladders containing gas, and red, yellow, brown or black material or fluids. No abnormality was noted in any of the surviving rats during necropsy.
Classification: Acceptable, Toxicity Category III.

152-11 An Acute Dermal Toxicity Study in Rabbits (MRID 431199-05)

Using a single dose, the acute dermal LD_{50} of ProCone® (ABG-3149) was determined to be greater than 2000 mg ABG-3149/kg and less than 5000 mg ABG-3149/kg in male and female New Zealand white rabbits. The animals (5 males and 5 females) that received a dose of 2000 mg ABG-3149/kg survived through the 14 day observation period and all appeared normal after day 5 of the study. A slight erythema was observed at the site of application in 4 out of 10 animals (one at day one and three rabbits at day three). Very slight erythema was noticed in one rabbit on day 7 and persisted in this rabbit until day 10. No edema or other dermal effects were observed at the site of application in any of the rabbits during the study.

Of the animals (5 males and 5 females) that received a dose of 5000 mg ABG-3149/kg, all died except 3 males (mortality rate 70%). All animals developed some sign of abnormality: fasciculation in two rabbits, pilo-erection in four rabbits, a decrease in the amount of feces in three rabbits, eyes partially closed in four rabbits, decrease in activity in six rabbits, and prostration in four rabbits was observed. Very slight erythema was observed at the site of application in two of the ten rabbits on day 1. No erythema was noted in the surviving animals after day 1 of the study. No edema or other dermal effects were observed at the site of application in any of the rabbits during the study. All surviving rabbits appeared normal after day 13 of the study. All animals except one gained weight. The animals that died lost weight prior to death. Necropsy in rabbits that died included tan material around the nose, mouth, and anogenital staining. No abnormal findings were noted at external examinations

were observed at the site of application in any of the rabbits during the study. All surviving rabbits appeared normal after day 13 of the study. All animals except one gained weight. The animals that died lost weight prior to death. Necropsy in rabbits that died included tan material around the nose, mouth, and anogenital staining. No abnormal findings were noted at external examinations during necropsy in any of the surviving rabbits in the 2000 and 5000 mg ABG-3149/kg.

Classification: Acceptable, Toxicity Category III.

152-12 Acute Inhalation Study in Rats (MRID 431199-06)

An acute (4-hour) inhalation toxicity study of ProCone (ABG-3149) in rats was determined to have LC_{50} equal to 3.644 mg/l. The aerosol was generated at 3.29 mg/l, 3.68 mg/l, and 4.85 mg/l. At 3.29 mg/l exposure, the mortality rate was 0%, while at 3.86, and 4.85 the mortality rates were 60% and 100% respectively. All animals survived the 3.29 mg/l exposure and gross necropsy after 14 days indicated that all animals had lungs mottled dark red. Observations noted for 3.29 mg/l exposure included: piloerection, activity decrease, nasal discharge, salivation, polyuria, and staggered gait in all animals up to two days after dosing. All cleared by day 3 after dosing. All animals gained weight at 7 and 14 days post-exposure.

Classification : Acceptable-Toxicity Category IV.

152-13 Primary Eye Irritation Study in Rabbits (MRID 430451-01)

A single dose (106 mg/0.1 ml) of ProCone (ABG-3149) produced corneal or iridal effects in both washed and unwashed eye groups. Conjunctival redness, chemosis and discharge were observed by the one-hour interval in the rabbits in both groups. Minimal conjunctival redness persisted in two rabbits in the unwashed group through day 10, and minimal redness persisted in one rabbit in the washed group through day 14. No ocular effects were observed in any of the rabbits on day 21 of the study. Washing of the eyes with water following test material administration resulted in a very slight difference in the incidence and persistence of ocular effects in the washed group when compared to the unwashed group. For the unwashed group the maximum mean total score, calculated according to the Draize method for grading ocular effects was 34.2, observed at the 72 hour interval. For the washed group, the maximum mean total score, calculated according to the Draize method for grading ocular effects was 27.3, observed on day 7 of the study.

Classification: Acceptable, Toxicity Category II.

152-14. Primary Dermal Irritation Study In Rabbits (MRID 430451-02)

A single dose (0.5 ml/1.06 g/l) of ProCone®(ABG-3149) produced well-defined erythema which was cleared by day 4. No edema or other dermal effects were observed in any of the rabbits during the study. The primary irritation index of ProCone®(ABG-3149) is 0.9.

Classification: Acceptable, Toxicity Category IV

DATA EVALUATION REPORT

Reviewed by: Freshteh Toghrol, Ph.D. BPPD
Secondary Reviewer: Sheryl K. Reilly Ph.D. BPPD SKR 2/15/95

STUDY TYPE:	Guideline No. 152-10; acute oral toxicity in rats
LIST/Chemical No.	List D/043801 Gibberellic acid
Case No.	4110
Caswell No.	467
MRID NUMBER:	431199-04
NAME; TEST MATERIAL:	ProCone® (ABG-3149) (Lot No. 78-087-BR)
SPONSOR:	Abbott Laboratories North Chicago, IL
STUDY NUMBER:	Ricerca Study No. 93-0159
TESTING FACILITY:	Ricerca, Inc. Department of Toxicology and Animal Metabolism, Painesville, OH
TITLE OF REPORT:	Acute Oral Toxicity (LD_{50}) Study In Rats With ProCone®(ABG-3149)
AUTHORS:	Steven K. Shults, B.A., Ann W. Brock, M.S., and James Laveglia, Ph.D.
DATE OF REPORT:	November 23, 1993
QUALITY ASSURANCE:	The test was performed under GLP standards. A Compliance Statement, signed by all three authors 11/23/93, 12/16/93, and 12/17/93 was submitted.
CONCLUSIONS:	In this study the acute oral LD_{50} of Procone® (ABG-3149) in rats is 2100 mg ABG-3149/kg with 95% confidence limits of 1510 to 2910 mg ABG-3194/kg.
CORE CLASSIFICATION:	This study is acceptable.
TOXICITY CATEGORY:	Tox category III

A. MATERIALS

Test Compound

Test material:	ProCone® ABG-3149
Lot No:	78-087-BR
Purity:	4.1% active ingredient Gibberellins A_4,A_7
Physical description:	Clear liquid
Storage conditions:	Room temperature
Stability:	Reported stable
Dose levels:	A single oral doses at dose 500, 2500, 3550, 5000 mg/kg In range finding 630, 1250, 2500, and 5000 mg ABG-3149/kg
Density:	1.06 g/ml

Controls

There were no control test materials or animals.

Test Animals

Species:	Albino Rabbits
Strain:	New Zealand White
Source:	Mohican Valley Rabbitry, Loudonville, Ohio.
Sex:	Male and female
Age at receipt:	Young adult
Weight (pre-fasted):	2.486 to 2.803 kg
No. animals:	10 (5 males and 5 females for each group)
Acclimation:	five or eleven days
Housing:	Individually housed
Food:	Purina Laboratory Chow #5002, ad libitum (except 18 hours before and 4 hours post-dosing)
Water:	Automatic watering system, ad libitum
Temperature:	Room temperature 67 to 75 °F, monitored and recorded twice daily.
Humidity:	40% to 70%
Photoperiod:	12 hours dark/12 hours light
Identification:	Cage cards and ear tags
Selection:	Random

B. TEST PERFORMANCE

Animals fasted:	16-20 hours overnight before dosing
Dosing:	oral, gastric intubation using syringe and ball tipped dosing needle, single dose
Observation period:	Fourteen days
Observation Frequency:	Twice daily

Body weight
Interval: Prior to fasting, immediately before dosing, and at 7 and 14 days post-exposure

C. RESULTS

Mortality

The mortality data for five male and five female rats per group are given in table 1.

Table 1. Mortality

Number Dead/Number Tested

Dose (mg/kg)	Males	Females	Combined
500	0/5	0/5	0/10
2500	1/5	4/5	5/10
3550	5/5	1/5	6/10
5000	5/5	5/5	10/10

Using a single oral dose by gavage, the material was administered at dosages of 500, 2500, 2550, and 5000 mg ABG-3149/kg to groups of five male and five female Sprague Dawley rats. The animals were observed for signs at 1, 2½, 4 hours following administrations on the day of dosing and daily thereafter for 14 days. The mortality rate was 0% for 500 mg ABG-3149/kg. The mortality rates for 2500, 3550, and 5000 mg ABG-3149/kg, were 50% (one male; second day post-dose and 4 female), 60% (5 male and one female), 100% (5 males and 5 female in first 4 hours one in first day after dosing were found dead). Necropsy in rats that died during the study indicated presence of red, yellow, brown, and black material or fluid in stomach, intestines, bladder. A distended stomach and distended small intestine were noted in one rabbit. No abnormality was noted in any of the surviving rats during necropsy.

Clinical Observations

In the 500 mg dose, rales were observed in one female rat and one male rat on day 1, post-dosing. A decrease in amount of feces were observed in two female rats on days 1, 2 after dosing. Lacrimation, ataxia, a decrease in the amount of feces, a decrease in activity, and prostration were noted in rats of both sexes in 2500, 3550, and 5000 mg ABG-3149/kg dose groups. These signs diminished by day 5 in the animals that survived.

Body weights

All surviving rats gained weight both 7 and 14 days after dosing.

1

Macroscopic postmortem Observations

No abnormal finding noted at external examinations during necropsy in the rats that died during the study was anogenital staining in the rats in the 2500 and 3550 mg ABG-3149/kg group. Brown material around the nose was observed in one rat and clear, colorless fluid around the eyes, and yellow material around the mouth was observed in another rat in the 3550 mg ABG-3149/kg group. Dried yellow/red material around the nose and mouth, and an enlarged abdomen was noted in one rat in the 50000 mg ABG-3149/kg group.

The only finding noted at external examinations during necropsy in the rats which survived to terminal necropsy was red material around the eyes of one male rat in the 2500 mg ABG-3149/kg group. This was considered an incidental finding probably not related to the test material. Findings noted at internal examinations during necropsy in rats which died during the study distended bladders containing gas, and red, yellow, brown or black material or fluid in the rats in the 2500, 3500, and 5000 mg ABG-3149/kg groups. Multiple black foci on the internal wall of the stomach were noted in one rat in each of the 2500 and 5000 mg ABG-3149/kg groups. The intestines had patchy red areas or were red in color in rats in the 2500 and 5000 mg ABG-3149 groups. The duodenum, jejunum, and ileum were yellow or green in color in rats in the 3550 mg ABG-3149/kg group and the jejunum was red in color in rats in the 5000 mg ABG-3149/kg group. the bladder was dark in color in rats in the 3550 mg ABG-3149/kg group and the bladder was red in color in rats in the 5000 mg ABG-3149/kg group. Dark red or red lungs were noted in rats in the 2500 and 5000 mg ABG-3149/kg groups, and patchy dark area on the lungs were noted in rats in the 3550 mg ABG-3149/kg group. A black or thickened spleen was observed in rats in the 2500 mg ABG-3149/kg group. The edges of the liver were black in one rat in 3550 mg ABG-3149/kg group. The kidneys were pale in one rat in the 5000 mg ABG-3149/kg group. The testicles were dark colored in one rat in the 3550 mg ABG-3149/kg group, and all the tissues were dark colored in one rat in the 2500 mg ABG-3149/kg group.

LD_{50} determination

The median acute oral LD_{50} in rats for ProCone®(ABG-3149) was 2100 mg/kg.

D. CONCLUSION

In this study the acute oral LD_{50} of Procone®(ABG-3149) in rats is 2100 mg ABG-3149/kg with 95% confidence limits of 1510 to 2910 mg ABG-3194/kg.

This study is acceptable, Toxicity category III.

DATA EVALUATION REPORT

Reviewed by: Freshteh Toghrol, Ph.D. BPPD
Secondary Reviewer: Sheryl K. Reilly Ph.D. BPPD SKR 2/15/95

Study Type:	Guideline No.152-11; acute dermal toxicity in rabbits
LIST/Chemical No.	List D/043801 Gibberellic acid
Case No.	4110
Caswell No.	476
MRID NUMBER:	431199-05
Name;TEST MATERIAL:	ProCone® (ABG-3149) (Lot No. 78-087-BR)
SPONSOR:	Abbott Laboratories North Chicago, IL
STUDY NUMBER:	
TESTING FACILITY:	Ricerca, Inc., Department of Toxicology and Animal Metabolism, Painesville, OH
TITLE OF REPORT:	Acute Dermal Toxicity Study In Albino Rabbits With Procone® (ABG-3149)
AUTHOR:	Steven K. Shults, B.A., Ann W. Brock, M.S., and James Laveglia, Ph.D.
DATE OF REPORT:	November 15, 1993
QUALITY ASSURANCE:	The test was performed under Good Laboratory Practice Standards. A Compliance Statement, signed by all three authors on 11/23/93, 12/16/93, and 12/17/93, was submitted.
CONCLUSIONS:	In this study, the acute dermal LD_{50} for ProCone (ABG-3149) in rabbits is greater than 2000 mg/kg and less than 5000 mg ABG-3149/kg.
CORE CLASSIFICATION:	This study is acceptable.
TOXICITY CATEGORY:	Tox category III

A. MATERIALS

Test Compound

Test material:	ProCone® ABG-3149
Lot No:	78-087-BR
Purity:	4.1% Gibberellins A_4A_7 as ai
Physical description:	Clear colorless liquid
Storage conditions:	Room temperature
Stability:	Reported stable
Dose levels:	2000 and 5000 mg/kg
Density:	1.06 g/ml

Controls

There were no control test materials or animals.

Test Animals

Species:	Albino Rabbits
Strain:	New Zealand White
Source:	Mohican Valley Rabbitry, Loudonville, Ohio
Sex:	Male and female
Age at receipt:	Young adult
Weight (day-1)	Males: 2.37-2.42 kg; Females: 2.46-2.48 kg
No. animals:	10 (1 group of 5/sex)
Acclimation:	6-7 days
Housing:	Individually housed
Food:	Lab Rabbit Chow HF (Purina No. 5326)
Water:	Automatic watering system, ad libitum
Temperature:	61-70° F, monitored and recorded twice daily.
Humidity:	40-60% monitored and recorded twice daily.
Photoperiod:	12 hours dark/12 hours light
Identification:	Ear tags imprinted with a unique No.
Selection:	Random

B. TEST PERFORMANCE

Preparation of Animal: Approximately 24 hours before dosing, the hair of each rabbit was clipped from the trunk with an electric clipper, so as to expose at least 10% of the body surface area. Care was taken to avoid abrading the skin during the clipping of the backs.

Dosing: The test material was applied directly onto the exposed skin and spread evenly over the entire area. A layer of 8 inch gauze was then secured at either end with Dermiform tape. A plastic restraining collar was placed around the rabbits neck and the animal was returned to the cage. After 24 hours, the wrappings were removed and the test site wiped free of excess test material.

Observaton period:	1, 2½, and 4 hours after dosing and twice daily from the day after dosing for fourteen days.
Scoring frequency:	1, 3, 7, 10, and 14 days of study.
Body weight interval:	Prior to dosing, on days 7 and 14 post-exposure.

C. RESULTS

Mortality

All animals that received 2000 mg ABG-3149/kg (5 males and 5 females) survived throughout the study. Of the animals (5 males and 5 females) that received a dose of 5000 mg ABG-3149/kg, 3 males survived (mortality rate 70%) throughout the 14 day observation period.

Body weight

All rabbits dosed at 2000 mg/kg exhibited body weight gain at 7 and 14 days after dosing. All surviving rabbits dosed at 5000 mg/kg also gained some weight 7 and 14 days after dosing. All rabbits were killed at fourteen days and subjected to gross necropsy. All abnormalities were recorded but no tissues were saved.

Clinical Observations

The 5 males and 5 females that received a dose of 2000 mg ABG-3149/kg survived throughout the 14 day observation period and appeared normal after day 5 of the study. The following observations may have been related to treatment: one female exhibited soft feces on day 1, two rabbits developed anogenital staining on day 3, a decrease in feces was noted in several rabbits on day 1, a decrease in activity was observed in most of the rabbits on the day of dosing, and fasciculations were noted in one rabbit. A slight erythema was observed at the site of application in 4 out of 10 animals (one rabbit at day one and three rabbits at day three). Very slight erythema was noticed in one rabbit on day 7 and persisted in this rabbit until day 10. No edema or other dermal effects were observed at the site of application in any of the rabbits during the study.

Of the 5 males and 5 females that received a dose of 5000 mg ABG-3149/kg, only 3 males survived throughout the 14 day observation period. All animals developed some sign of abnormality including fasciculation in 2, pilo-erection in 4, a decrease in the amount of feces in 3, eyes partially closed in 4, decrease in activity in 6, and prostration in 4 rabbits. All surviving rabbits appeared normal after day 13 of the study and gained weight except one. The animals that died lost weight prior to death. Necropsy in rabbits that died included tan material around the nose and mouth, and anogenital staining. No abnormal findings were noted at external examinations during necropsy in any of the surviving rabbits in the 2000 and 5000 mg ABG-3149/kg. Very slight erythema was observed at the site of application in two of the ten rabbits on day 1. No

11

erythema was noted in the surviving animals after 1 day of study. No edema or other dermal effects were observed at the site of application in any of the rabbits during the study.

Using a single dose, the acute dermal LD_{50} of ProCone® (ABG-3149) was determined to be greater than 2000 mg ABG-3149/kg and less than 5000 mg ABG-3149/kg in male and female New Zealand white rabbits.

Necropsy

Findings noted at external examinations during necropsy in rabbits in the 5000 mg ABG-3149/kg group which died during the study included tan material around the nose and mouth, and anogenital staining. No abnormal findings were noted in any of the surviving rabbits in the 5000 mg ABG-3149/kg group or the 2000 mg ABG-3149/kg group.

Findings noted at internal examinations during necropsy in rabbits in the 2000 mg ABG-3149/kg group included multiple round white worms in the cecum of one rabbit, one or more ovarian cysts in five rabbits, mottled lungs in two rabbits, and kidneys pale in color in one rabbit. Roundworms are not considered to be pathogenic in rabbits and therefore were not considered to have any adverse effect on the results of the study. The ovarian cysts are a common finding and were not considered treatment related.

Findings noted at internal examinations during necropsy in rabbits in the 5000 mg ABG-3149/kg group which died during the study included a mottled, pale or dark liver, mottled or brown kidneys, black or dark small spleen, dark red lungs with black multiple foci. A distended stomach and distended small intestine were noted in one rabbit. An extremely thin bladder was found in another rabbit. This finding was most likely not related to treatment due to single incidence in this group of animals. No abnormal findings were observed in any of the rabbits which survived to terminal necropsy in the 5000 mg ABG-3149/kg group.

LD_{50} Determination

The estimated acute dermal LD_{50} in rabbits for Procone was greater than 2000 mg ABG-3149/kg and less than 5000 mg ABG-3149/kg.

D. CONCLUSION

Acute dermal exposure of rabbits to 2000 mg ABG-3149/kg resulted in 0% (0/10) mortality; therefore, the acute oral LD_{50} is greater than 2000 mg ABG-3149/kg and less than 5000 mg ABG-3149/kg in male and female New Zealand white rabbits.

This study was acceptable, toxicity category III.

DATA EVALUATION REPORT

Reviewed by: Freshteh Toghrol, Ph.D. BPPD
Secondary Reviewer: Sheryl K. Reilly Ph.D. BPPD SKR 2-15-95

STUDY TYPE: Guideline series 152-12; acute inhalation toxicity in rats

List/CHEM No: List D/043801

MRID NUMBER: 431199-06

TEST MATERIAL: ProCone ABG-3149 (Lot No. 78-087-BR)

SPONSOR: Abbott Laboratories
North Chicago, IL

STUDY NUMBER: Stillmeadow Study No. 0323-93

TESTING FACILITY: Stillmeadow, Inc. North Chicago, IL

TITLE OF REPORT: Procone (ABG-3149)
Acute Inhalation Toxicity Study In Rats

AUTHOR: Mark S. Holbert

REPORT DATE: 11/2/93

QUALITY ASSURANCE: The test was performed under Good Laboratory Practice Standards. A Compliance Statement, signed 12/16/93, was submitted.

CONCLUSIONS: The 4-hour acute inhalation LC_{50} (aerosol) in rats for ProCone (ABG-3149) is equal to 3.644 mg/l with 95% confidence limits of 3.477 to 3.820 mg/l.

CLASSIFICATION: Acceptable

TOXICITY CATEGORY: IV

A. MATERIALS

Test Compound

Test material: Gibberellic acid active ingredient (ProCone ABG-3149).

Source:	Abbott Laboratories
Lot No.:	78-087-BR
Purity:	4.2% ai (4.2% Gibberellins A_4,A_7 (GA_4,A_7)
Physical description:	Clear liquid
Storage:	Room temperature in closed glass containers
Stability:	Reported stable
Exposure level:	Target: 3.29, 3.68, and 4.85 mg/l ProCone (ABG-3149) aerosol
Exposure period:	Single 4-hour inhalation in rats
Observation period:	14 days

Controls

There were no control test materials or animals.

Test Animals

Species:	Rat
Strain:	HED:SD
Source:	Harlan Sprague Dawley, Inc. Houston, TX.
Sex:	Male and female
Age at exposure:	Young adult
Mean body weight (at exposure):	232-287 g for males; 205-223 g for females
No. animals:	Fifteen males and fifteen females
Temperature:	19-24° C (target range)
Humidity:	64-93% (target range)
Photoperiod:	12 hours dark/12 hours light
Food:	Purina Mills, Inc. St. Louis, MO
Water:	Tap water, ad libitum (except during exposure)
Acclimation:	11-20 days
Housing:	Individually housed
Identification:	Ear tags
Selection:	Random

B. TEST PERFORMANCE

Inhalation Chamber

A 200 liter New York University design, stainless steel, dynamic flow inhalation chamber was used. A maximum of 10 animals were exposed during any given exposure period. Animals at the 3.68 and 4.85 mg/l levels were washed after the exposure in an attempt to remove excess test material. All animals were returned to their stock laboratory cages at the termination of the exposure period.

Generation of test atmospheres

Due to the physical nature of the test material ProCone (ABG-3149), a concentration greater than 2.19 mg/l that would contain at least 25% of particles under 1.0 micron could not be generated.

The aerosol was generated at the 3.29 mg/l level by a pressure operated Spraying System Company air atomizer (¼ JSS) with a nebulizing ball attached, which aspirated the test material directly from its container, and then elutriated the resulting aerosol through a baffling chamber. The concentrated aerosol was then diluted with dried and filtered ambient air and drawn into the exposure chamber. The aerosol was generated at 3.68 and 4.85 mg/l levels by pumping the test material into a pressure operated Spraying System Company air atomizer (¼ JSS) and then spraying the resulting aerosol directly into the inhalation chamber. Air flow into the chambers was maintained through the use of a calibrated critical orifice at a rate of 20.1-29.3 air changes per hour. Air flow was recorded at 30 minute intervals during the exposure period by measuring the pressure drop through the calibrated critical orifice, using a Magnehelic gauge, and was sufficient to ensure an oxygen content of at least 19% of the exposure atmosphere. The air from the exposure chamber was cleaned by a hepa and charcoal filter unit. Temperature and humidity were recorded at 30 minute intervals during the exposure period from a Taylor wet bulb/dry bulb hygrometer located in the exposure chamber. The concentration of test material in the exposure atmosphere was determined analytically once per hour (taken from the breathing zone of the animals), and nominally at the end of each exposure. The analytical determination was made using a Beckman System Gold HPLC with Autoinjector. The nominal concentration was determined by dividing the loss in weight of the test material after each exposure by the total volume of air which passed through the chamber.

Analytical determinations and Chamber monitoring

Particle size (taken from the breathing zone of the animals) was determined twice during each exposure, using an Andersen cascade impactor, at a rate of 28.3 l/minute for a duration of 1-2 minutes. The mass median aerodynamic diameter and percentage of the mass of the particles under 1.1 microns is calculated from this data.

Chamber operating parameters for all three test concentration were given. These parameters were virtually identical and are as follows: temperature (73-76 °F and 23-24 °C), percent relative humidities (55%-63%), air flow 97.6. Concentration determination and calculation of nominal concentration was given for each concentration

Table 1. Atmospheric Concentration (Aerosol) and Particle Size Distribution of the Test Material *

Test Material Concentration mg/l	Mean Exposure Concentration (mg/l)	Nominal Concentration mg/l
3.29	3.87 ± 0.5	12.5
3.68	3.676± 0.24	62.6
4.85	4.851± 0.28	165.2

* Data were extracted from pages 16 and 17 of the study report. (Stillmeadow, Inc. 0323-93, Submitted by Abbott, MRID No. 431199-06).

Observations

Exposure period: Single 4-hour exposure

Observation period: 14 days

Observation frequency: 0.5, 1.0, 2.5, 4.5, 6.0 hours following exposure; and daily throughout the 14-day observation period.

Body weight interval: Prior to exposure and 7 and 14 days post-exposure.

C. RESULTS

Mortality

All animals in the 4.85 mg/l Procone (ABG-3149) inhalation concentration were found dead within 2 days of the treatment. Six (3 males and three female) animals in the 3.68 mg/l Procone (ABG-3149) inhalation concentration were found dead in the first week after the treatment. All animals survived the 3.29 mg/l Procone (ABG-3149) inhalation concentration for a test period of 14 days. LC_{50} = 3.644 mg/l ProCone (ABG-3149) with 95% confidence limits of 3.477 to 3.830 mg/l was calculated.

Number of dead/Number of treated

Test Material Conc. (mg/l)	Males	Females	Male+Females Combined
3.29	0/5	0/5	0/10
3.68	3/5	3/5	6/10
4.85	5/5	5/5	10/10

* Data were extracted from page 10 of the study report (Stillmeadow, Inc. Study No. 0323-93, MRID No. 431199-06).

Clinic, Pharmacologic, and Toxicologic Signs

Observations noted for 3.29 mg/l exposure: piloerection, activity decrease, nasal discharge, salivation, polyuria, and staggered gait in all animals up to two days after dosing. All cleared by the third day after dosing. Observations noted for 3.68 mg/l exposure: piloerection, activity decrease, fur coated with test material, polyuria in all animals up to two days after dosing. All cleared by the fourth day after dosing in animals that survived. Observations noted for 4.85 mg/l exposure: piloerection, activity decrease, and fur coated with test material in all animals up to one day after dosing. All animals died within 2 days following dosing.

Body weights

All animals at 3.29 mg/l exposure survived the exposure and gained weight (male + female) at 7 and 14 days after exposure. All animals that survived (40%) at 3.68 mg/l exposure indicated a slight body weight gain. However, all animals that were exposed to 4.85 mg/l and survived indicated a slight body weight loss.

Organ weights

Organ weight data were not provided in the study report.

Gross necropsy

All animals survived the 3.29 mg/l exposure. Gross necropsy after 14 days indicated that all animals (male + female) had lungs mottled dark red. Observations noted for 3.68 mg/l exposure in treated rats were as follows: all animals that survived exposure (40%) had lungs mottled dark red; all animals that did not survive were found dead in the first 4-6 hours or first day showed polyuria, nasal discharge and salivation; test material on fur; lungs red and slightly swollen. Observations noted for 4.85 mg/l exposure in treated rats were as follows: All animals were found dead in the first few hours or first day of exposure and showed the following signs: polyuria, nasal discharge and salivation; test material on fur; lungs red and slightly swollen, testes drawn into abdominal cavity; gastrointestinal tract distended with gas, and some lungs were speckled red and were swollen to twice their normal size.

D. CONCLUSIONS

The acute 4-hour inhalation of ProCone (ABG-3149) as a liquid aerosol has a calculated LC_{50} of 3.644 mg/l in rats, with 95% confidence limits and the slope function (S) with 95% confidence limits for Procone (ABG-3149), when administered undiluted as an aerosol to albino rats.

Classification: Acceptable. Toxicity Category IV.

DATA EVALUATION REPORT

Reviewed by: Freshteh Toghrol, Ph.D. BPPD
Secondary Reviewer: Sheryl K. Reilly Ph.D. BPPD 5/2-2-15-95

Study Type:	Guideline series 152-13; primary eye irritation in rabbits
LIST/Chemical No.	List D/043801 Gibberellic Acid
Case No.	4110
Caswell No.	467
MRID NUMBER:	430451-01
Name;TEST MATERIAL:	ProCone (ABG-3149) (Lot No. 78-087-BR)
SPONSOR:	Abbott Laboratories North Chicago, IL
STUDY NUMBER:	Ricerca Study No 93-0161
TESTING FACILITY:	Ricerca, Inc. Department of Toxicology and Animal Metabolism Painesville, OH
TITLE OF REPORT:	Primary Eye Irritation Study In Albino Rabbits With Procone (ABG-3149)
AUTHOR:	Steven K. Shults, B.A., Ann W. Brock, M.S., and James Laveglia, Ph.D.
DATE OF REPORT:	November 15, 1993
QUALITY ASSURANCE:	The test was performed under Good Laboratory Practice Standards. A Compliance Statement, signed by all three authors 11/15/93, 11/23/93, and 11/23/93 was submitted.
CONCLUSIONS:	ProCone (ABG-3149) produced corneal or iridal effects in both unwashed and washed eye groups. Conjunctival redness, chemosis and discharge were observed by the 1-hour interval in the rabbits in both groups. Minimal conjunctival redness persisted in two rabbits in the unwashed group through day 10 and, in one rabbit in the washed group through day 14. No ocular effects were observed in any of the rabbits on day 21 of the study. Washing of eyes with water following test material administration resulted in a very slight difference in the incidence and persistence of ocular effects in the washed group when compared to the unwashed group.
CORE CLASSIFICATION:	Acceptable.
TOXICITY CATEGORY:	Tox category II

A. MATERIALS

Test Compound

Test material:	ProCone (ABG-3149)
Lot No.:	78-087-3149
Purity:	4.1% Gibberellins A_4A_7
Physical description:	Clear colorless liquid
Storage conditions:	Room temperature
Dose levels:	0.1 ml
Density:	1.06 g/ml

Controls

There were no control test materials or animals.

Test Animals

Species:	Albino Rabbits
Strain:	New Zealand White
Source:	Mohican Valley Rabbitry, Loudonville, Ohio
Sex:	Male and female
Age at receipt:	Young adults
Weight (pre-fasted):	Ranged from 2.250 to 2.569 Kg.
No. animals/dose:	Nine (unwashed eyes: 3 males, 3 females; washed eyes: 3 females)
Acclimation:	21 days
Housing:	Individually housed
Food:	Lab Rabbit Chow HF (Purina #5326), ad libitum
Water:	Automatic watering system, ad libitum
Temperature:	Room temperature, monitored and recorded twice daily.
Photoperiod:	12 hours dark/12 hours light
Identification:	Cage cards and ear tags
Selection:	Random

B. TEST PERFORMANCE

Dosing:	A single dose of 0.1 ml undiluted ProCone was introduced into the lower conjunctival sac of the right eye of each animal. The upper and lower lids were gently held together for a second to prevent loss of material. The left eye was used as the control.
Observation:	Three days (approximately 1, 24, 48, and 72 hours after treatment and on days 4, 7, 10, 14, 21 of the study. An examination was performed utilizing an ultraviolet light source, of all treated eyes following staining with 2% sodium fluorescein solution to check for the presence of corneal ulceration. Just prior to the test application, the eyes were examined again, but without fluorescein. Animals showing preexisting conditions were not used.

C. RESULTS

Clinical observations

All animals survived the study period. All animals were killed at termination of the study by an intravenous injection of a euthanasic agent, Sleepaway®. Necropsies were not conducted.

Ocular Observation

All six animals exhibited irritation consisting primarily of conjunctival irritation, with scores of 1 or 2 for redness, chemosis or discharge. Three animals also had slight iridial changes.

Unwashed eyes:

Corneal opacity was observed in one rabbit at one hour interval, in three rabbits at 24-hours interval, and in six rabbits from the 48 hours interval through day 4. Corneal opacity persisted in one rabbit through day 7. No corneal opacity was observed in any of the rabbits after day 7 of the study. Corneal epithelial effects involving up to approximately 25% of the corneal surface persisted in one rabbit through day 7 of the study. Five of the six animals with unwashed eyes were free of all ocular irritation by 24 or 48 hours, with the remaining animal free of irritation by 72 hours (both the test and control eyes had a discharge score of one at 48 hours).

Washed Eyes:

Corneal opacity was noted in two rabbits on day 7 and persisted in these tqwo rabbits through day 10. No. corneal opacity was observed in any of the rbbits after day 10 of the study. Corneal vascularization also was observed in this group up to 25% was oberved in two rabbits on day 10 and up to 5% of the corneal vascularization persisted in one rabbit through day 14. Corneal epithelial effects involving up to approximately 25% of the corneal surface persisted in two rabbits through day 7 of the study. This effect was noted in two rabbits on day 7 and persisted in theser two rabbits through day 10 of the study.

D. CONCLUSION

A single dose (106 mg/0.1 ml) of ProCone® (ABG-3149) produced a Conjunctival redness which persisted in two rabbits from the unwashed group through day 10 after dosing and in one rabbit in the washed group through day 14. No ocular effects were observed in any rabbits after day 21. For the unwashed group, the maximum mean total score, calculated according to the Draize method for grading ocular effects was 34.2, observed at the 72 hour interval. For the washed group, the maximum mean total score by the Draize method for grading ocular effects was 27.3, on day 7 of the study.

Classification: Acceptable, Toxicity Category II.

Reviewed by: Freshteh Toghrol, Ph.D. BPPD
Secondary Reviewer: Sheryl K. Reilly Ph.D. BPPD 2-15-95

Study Type: Guideline No. 152-14; primary dermal irritation study in rabbits list

Chemical No. List D/043801 Gibberellic acid

Case No. 4110

Caswell No. 467

MRID NUMBER: 430451-02

Name;TEST MATERIAL: ProCone®(ABG-3149) (Lot No. 78-087-BR)

SPONSOR: Abbott Laboratories
North Chicago, IL

STUDY NUMBER: Ricerca Study No. 93-0162

TESTING FACILITY: Ricerca, Inc. Department of Toxicology and Animal Metabolism
Painesville, OH

TITLE OF REPORT: Primary Dermal Irritation Study In Albino Rabbits
With ProCone®(ABG-3149)

AUTHOR: Steven K. Shults, B.A., Ann W. Brock, M.S., and James Laveglia, Ph.D.

DATE OF REPORT: 12/2/93

QUALITY ASSURANCE: The test was performed under Good Laboratory Practice Standards. A Compliance Statement, signed by all three authors 12/7/93, 12/7/93, and 12/2/93, was submitted.

CONCLUSIONS: The primary skin irritation index in this study is 0.9 for ProCone® (ABG-3149) in rabbits. This is a mild or slight irritant or erythema.

CORE CLASSIFICATION: This study is acceptable.

TOXICITY CATEGORY: Tox category IV.

A. MATERIALS

Test Compound

Test material:	ProCone®(ABG-3149)
Lot No.:	78-087-BR
Purity:	4.1% Gibberellins A_4A_7
Physical description:	Clear colorless liquid
Storage conditions:	Room temperature
Stability:	Reported stable
Dose levels:	0.5 ml/site
Density	1.06 g/ml

Controls

There were no control test materials or animals.

Test Animals

Species:	Albino Rabbits
Strain:	New Zealand White
Source:	Mohican Valley Rabbitry, Loudonville, Ohio
Sex:	Male and female
Age at receipt:	Young adult
Weight (day-1)	2.533 to 2.794 kg
No. animals	6 (2 groups of 3/sex)
Acclimation:	16 days
Housing:	Individually housed
Food:	Lab Rabbit Chow HF (Purina #5326)
Water:	Automatic watering system, ad libitum
Temperature:	Room temperature, monitored and recorded twice daily.
Humidity:	Not given: monitored and recorded twice daily.
Photoperiod:	12 hours dark/12 hours light
Identification:	Ear tags
Selection:	Random

B. TEST PERFORMANCE

Preparation of Animal:	Approximately 24 hours before dosing, the hair of each rabbit was clipped from the trunk with an electric clipper. 0.5 ml (530 g) of ProCone®(ABG-3149) was applied directly onto the exposed skin, beneath layers of gauze patch, and was secured with non-irritating tape. The plastic restraining collar was placed around the rabbit's neck and the animal was returned to its cage.
Observation period:	At 30 - 60 minutes, 24 hours, 48 hours, and 72 hours, and the 4th day of exposure, the test site was evaluated for erythema, eschar, and edema, according to the Draize system.
Body weight interval:	2.533 to 2.794 kg

22

C. RESULTS

Mortality

All animals (3 males and 3 females) survived throughout the study.

Body weight

Not reported.

Evaluation of Dermal Irritation:

At each interval all sites were evaluated for erythema and edema or other evidence of dermal irritation. Very slight to well-defined erythema was observed at the site of application in five of six animals from the 30 to 60 minute interval through the 24 hour interval. Very slight erythema was noted in three rabbits at the 48 hour interval and persisted in these three rabbits through the 72-hours interval. No erythema was noted in any of the rabbits on day 4. No edema or other dermal effects were observed in any of the rabbits during the study.

D. BPPD CONCLUSION

The test material, 0.5 ml (1.06 g/l) of ProCone®(ABG-3149), produced well defined erythema which was cleared by day 4. No edema or other dermal effects were observed in any of the rabbits during the study. The primary irritation index of ProCone®(ABG-3149) is 0.9.
Classification: Acceptable, Toxicity Category IV

Guideline No. 152-15; Dermal Sensitization Study:

The registrant did not submit Guideline No. 152-15; dermal sensitization study.

BPPD Comments

BPPD waives the data requirement for a dermal sentization study (Guideline No. 152-15) for this end-use product. However, any observed incidents must be reported.

cc: R. Sjoblad, F. Toghrol, BPPD Subject File.
F. Toghrol, F.T.CS#1: (703)308-7014: 1/31/95.