US EPA ARCHIVE DOCUMENT

(8-12-92)

MEMORANDUM:

Subject: EPA File Symbol/EPA Reg. No.:62637-A

From: Lucy D. Markarian, Biologist 8/4/92
Precautionary Review Section
Registration Support Branch
Registration Division (H7505C)

To: Phil Hutton, PM 18
Insecticide-Rodenticide Branch
Registration Division (H7505C)

Thru: Thomas C. Ellwanger, Section Head E 8/12/92
Precautionary Review Section
Registration Support Branch
Registration Division (H7505C)

Applicant: Becker Microbial Products, Inc.
9464 N.W. 11 th Street
Plantation, Florida 33322

FORMULATION FROM LABEL:

Active Ingredient(s):: Bacillus Thuringiensis, Subspecies Kurstaki 10,750 IU/mg	% by wt. 2.15 %
Inert Ingredient(s):	
.....	97.85 %
Total:	100.00 %

BACKGROUND

Two sets of tests have been submitted to support the registration of BMP 123(48 LC) high potency larvicide under the registration symbol 62637-A. The formulation is a microbial pesticide containing Bacillus thuringiensis var. Kurstaki in an aqueous suspension with 10,750 IU/mg.

The first set of the presented tests include all the required assays for registration, but are conducted with a solid test material identified as BMP Technical Powder with 110 000 IU/ mg.

The second set consists of two tests: oral and inhalation tests conducted with the material to be registered under EPA 64637-A

RECOMMENDATION

The tests conducted with the powdered product BMP technical cannot, at this time, support the registration of BMP 123, because the composition of the tested product is not known. The dermal toxicity, eye and dermal irritation, dermal sensitization and acute inhalation tests are considered supplementary data. The tests involving the skin are considered supplementary due to the fact that instead of moistening the test material the laboratory has in effect diluted it to more than 1:1. The effect of this way of "moistening" is that while the solids in the test material may be limited to the test area on the skin, the soluble parts are effectively diluted and not tested at the strength that they need to be. The soluble faction is dispersed into the gauze patch and removed from full contact. There is the possibility of the paste (made out of the test material) oozing out and not effectively in contact with the skin also, depending on the consistency. If the composition of the test material is presented, including the inerts (as some fermentation byproducts are most often soluble), and it is shown that the liquid used per application can be justified, then dermal toxicity and irritation tests may be upgraded. A new eye irritation test needs to be submitted. The inhalation test in this set of studies is rejected, primarily because the particle size is not fine enough to be respirable to the test model. The average MMAD is greater than 5 um. A new sensitization test need not be submitted, because the most recent guidelines for microbial pesticides do not include this study reqiusite for registration. However, sensitization incidents must be reported.

The tests conducted with the formulation BMP 123 (48 LC) are acceptable support for the registration of EPA 62637-A as guideline data.

PRS recommends that Backer Microbial Products provide the Following:

1-Ingredients of the test material BMP concentrate, and their percentages present in the formulation.

2-The reasoning for the excessive moistening of the test material during the tests requiring dermal application.

3-Present a new eye study conducted with the formulation BMP 123 (49 LC). The new study by virtue of being more dilute may be in category IV toxicity, and the evaluations of the eyes in the presented study are equivocal. PRS recommends the use of the Draize scale with no modifications. Discharge must be included in the evaluations.

If the inerts of the tested product (BMP technicl powder) are substantially similar to the inerts in the formulation BMP 123, and there is justification for moistening the test material to the extent that it has been moistened, then the dermal toxicity and dermal irritation tests may be acceptable as support. If this is not possible, new dermal toxicity and dermal irritation studies need to be submitted using BMP123 (48 LC).

The following is the rationale behind the rating of the tests conducted with BMP technical powder:

Oral Toxicity- Core minimum

1-The source of the animals is not specified.
2-Judged by the weights, the males were not truly young adults, at fasting weight all were over 300 grams.

Dermal Toxicity- Supplementary

1-An animal weighing 2.5 K would require 5.0 g of test material. This moistened with 9 ml of saline would be equivalent to a 1: 1.8 dilution. The guidelines call for using the test material moistened and not diluted. The form in which the product was tested does not reflect the actual toxicity of the formulation. The test may support a more dilute formulation.

2-The source of the animals is not specified.

3-60 % of the animals were above the specified weight range by the guidelines.

Inhalation Toxicity - Supplementary

1-The average particle size (5.07 um) is too large to be respirable to the test model. Percentage of particles under 2 um ranges from 11.0 -18.7 %. There is not enough uniformity in the particle size during exposure. Milling the test material prior to packing the dust generator canisters might have improved the particle size of the aerosol.

2-It is recommended by the guidelines that fiber glass filters be used. Charcoal filters were utilized.

3-The males weighed more than the recommended weight range of 200 - 300 grams.
4-The source of the animals was not specified as required by the guidelines.

Eye Irritation - Supplementary

1-PRS considers grade 1 opacity to be a positive effect. Therefore all the observed grade 1 opacity that is considered as unremarkable by the laboratory is positive for eye irritation.

2-Whenever there is dye retention, it is a sign of at least grade 1 opacity, if there was no dye retention before the test. If there is staining of the cornea, however light, at an observation time after an interval where definite opacity and dye retention has been observed, it is an indication that the cornea has not completely healed, and needs to be noted.

3-According to the guidelines it is not sufficient to just state grade 1 opacity. The area of opacity must also be specified. Furthermore, it is not clear how grade 1 opacity in such a large area as depicted by the sketch, can persist as grade 1 past 72 hrs. PRS is concerned about the accuracy of these readings. The same kind of ambiguity is present when grade 1 redness lasts for more than a week. There is no good probability for this. Grade 1 redness at 1 hour is not likely to last for more than a week. The test cannot be accepted only because there was no dye retention at 7 days. This is not always a sign of absence of opacity, because the eye heals from the surface inward, the absence of dye retention may or may not indicate opacity. If the corneal epithelium has healed the eye will not stain, but may still have opacity.

Dermal Irritation - Supplementary

1-By using 0.7 ml of saline per patch the test material was tested at a 1:1.4 dilution. The guidelines call for moistening of the test material, but not for dilution. The test does not define the dermal irritation potential of the test material, but may be able to support a more dilute formulation.

2-The source of the animals is not given. According to the guidelines this must be included in the report

Dermal Sensitization - supplementary

1-The choice of induction and elicitation concentration is arbitrary. Induction is conducted at less than 50 % without really knowing if the test material is irritating or not. The rabbit dermal irritation test was conducted at the same (1:

1.4) dilution and could not have given guidance. Also there is a difference in irritation between a six hour and a four hour exposure, even with species differences. The guidelines call for moistening of the test material when applied in solid form. The way it was applied in this test, it was diluted. It was not completely non irritating; therefore, it was possible to be tested at a higher concentration and perhaps cause irritation. In this sense it was not induced at the lowest irritating concentration.
2-Challenge was at the same concentration as elicitation, this was not the highest non irritating level either.
Buehler defines this concentration as that concentration that results in two grades of 0 and two of ± when tested in four guinea pigs. This was not demonstrated.

The principle on which sensitization assays work is that most assays induce at a slightly irritating concentration and elicit at a remote site at a lower non irritating concentration. If no reaction is observed with the lower challenge concentration, sensitization is not established, provided that the challenge concentration is not so low as to pass the threshold beyond which elicitation is not possible . This is the reason it is important to determine the highest nonirritating concentration for challenge. Induction and elicitation cannot be at the same concentration unless induction is at 100 %, and it was demonstrated to be completely nonirritating.

3-The guinea pigs were not restrained. Buehler states that restraining the animals is essential to the success of the test, because it improves contact between test material and skin and helps to hydrate it.

4-There were no naive controls. The base for comparison in deciding whether the formulation is a sensitizer are the reactions in the naive control animals and not the positive controls.
The only time when naive controls can be eliminated is when it is demonstrated that 100 % test material is unequivocally non irritating. This was not the case.

5-No information about the positive controls is included in the report. It is not known if the test was run within an acceptable time frame (within three months of the controls), nor were the actual results of the positive controls included. PRS prefers to reach conclusions independently, based on actual results.

6-PRS encourages the use of the Buehler grading system in a Buehler test. This avoids much confusion. It has a quantal approach and for general purposes is not graded like the Draize scale.

LABELING

The label can be more successfully recommended at the completion of the requirements. At the present there are only two acceptable tests and these alone cannot define the precautionary language for this product.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (§ 81-1)

Product Manager:18 **Reviewer:** L. Markarian
MRID No.:418266-02 **Report Date:**3/23/88
Testing Facility: Cosmopolitan Safety Evaluation Inc.
Report No.A1824
Author(s): Geoffrey Robbins
Species: Rat, Sprague Dawley
Age: Young adult
Weight:229 - 309 g
Source: Unspecified
Test Material:BMP Technical (110,000 IU/mg) Lot 28 brown powder
Quality Assurance (40 CFR §160.12): Included

Conclusion:

1. **The estimated LD_{50} is** > 5000 mg/kg
2. **Tox. Category:**IV **Classification:**Core minimum

Procedure (Deviations from §81-1):
Fasted animals from an unspecified source were intubated with the test material as a 50 % mixture in distilled water (20 g qs to 40 ml). Observations were at 1, 3, and 5 hours after treatment and daily thereafter. Body weights were recorded at initiation and on days 7 and 14. Necropsy was performed on all animals.

Results:

Dosage mg/kg	(Number Killed/Number Tested)		
	Males	Females	Combined
5000	0/5	0/5	0/10

Symptoms & Gross Necropsy Findings:

There was no mortality, symptom of toxicity or sin of gross pathology.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (§81-2)

Product Manager:18
MRID No.: 418266-03
Testing Laboratory: Cosmopolitan Safety
Author(s): Geoffrey Robbins
Species: Rabbit, New Zealand White
Weight:2.5 - 3.5 K
Source: Unspecified
Test Material:BMP Technical Powder 110,000 IU/mg, Lot 28, powder
Quality Assurance (40 CFR §160.12): Included

Reviewer: L. Markarian
Report Date:3/28/88
Report No.:B1824

Summary:

1. **The estimated LD_{50} is** > 2.0 g/kg

2. **Tox. Category:** III **Classification:**supplementary upgradeable

Procedure (Deviation From §81-2):

The test material was applied moistened with saline (approximately 9 ml per animal) to the clipped intact skin of the animals. The sites were covered with gauze pad and the trunks of the animals were wrapped with perforated plastic sheeting secured with tape. At 24 hrs the wrappings were removed and the sites wiped with clean, moist paper towels. Observations were frequent during the day of application and daily thereafter. There were twice daily mortality checks on week days. The dermal reactions were evaluated on days 1, 3, 7 and 14 according to Draize.

Results:

Reported Mortality

DOSAGE g/K	(NUMBER KILLED/NUMBER TESTED)		
	Males	Females	Combined
2.0	0/5	0/5	0/10

Symptoms & Gross Necropsy Findings:

No mortality, signs of toxicity or gross pathology were observed. Grade 1 erythema and edema are reported on days 1-3.

8

9.

DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (§81-3)

Product Manager:18 **Reviewer:** L. Markarian
MRID No.: 418266-04 **Report Date:**4/24/88
Testing Laboratory: Cosmopolitan Safety **Report No.:**C1824
Author(s): Geoffrey Robbins
Species: Rat, Sprague Dawley
Weight:M 240 - 340 g, F 200 - 300 g
Source: Unspecified
Test Material: Bmp Technical Powder 110,00 IU/mg, Lot 28, Powder
Quality Assurance (40 CFR §160.12): Included

Summary:

1. **The estimated LC_{50} is**

2. **Mean Concentration:**

3. **Tox. Category:** **Classification:**supplementary

Procedure (Deviation From §81-3):

Two groups of ten animals were used for the test. A group exposed to the test atmosphere and a control group exposed to air only.

Exposure was in a semicylindrical, 47.4 L chamber. The test material was introduced from a portal at the top and exhausted from the bottom with a vacuum pump.

The test atmosphere was generated using a Wright dust generator and Gast Air pump supplied the air(3.0 psi). A discharge tube delivered the aerosol from the dust generator to the chamber. Exhaust was maintained at 10 lpm as measured by a flowmeter. In addition to the 240 minute exposure time was allowed for equilibration (15 Minutes).

Chamber concentrations were measured gravimetrically using an "aerosol analysis monitor" that consisted of two superimposed filters with activated charcoal between them. Sampling was from the breathing zone at $\pm$ 1 lpm for no more than 10 minutes.

Particle size determination was made four times during the exposure, using a cascade impactor (Casella).

The chamber temperature and humidity were monitored and recorded at 30 minute intervals. Mean air flow was constant at 10 lpm

Observations were hourly during the exposure. Animals were observed after being taken out of the chamber, at 1, 3, and 5 hour and daily thereafter. There were twice daily mortality checks on week days. Body weights were recorded at initiation and on days 2, 3, 4, 7, and 14.
Necropsy was performed on all animals.

Results:

Chamber Concentrations	Test				Control
Gravimetric mg/kg	1.5				0
Range	1.44 - 1.70				
MMAD um	I	II	III	IV	
	4.3	6.5	5.0	4.5	
Average um	5.07				
GSD um	2.3	3.1	1.9	1.9	
% < 2 um	17.1	14.0	11.0	18.7	
Chamber temperature ^{0} F	68 - 76				68 - 76
Humidity %	47 - 67				66 - 82
Air flow lpm	10				10
Mortality					
Males	0/5				0/5
Females	0/5				0/5
Combined	0/10				0/10
Signs of Toxicity During exposure	Dorsum covered with test material				None
Post exposure	Brown stain				None
Day 3 - 14	Normal				Normal
Necropsy Findings	None				None

DATA REVIEW FOR ACUTE EYE IRRITATION TESTING (§81-4)

Product Manager:18
MRID No.: 418266-05
Testing Laboratory: Cosmopolitan Safety
Author(s): Geoffrey Robbins
Species: Rabbit, NewZealand White
Sex: 4 M and 2 F
Weight:2.0 - 3.5 K
Source: Not specified
Dosage:0.1 g
Test Material:BMP Technical Powder 110,000 IU/mg, Lot 28, Powder
Quality Assurance (40 CFR §160.12): Included

Reviewer: L. Markarian
Report Date:3/23/88
Report No.:D1824

Summary:

1. **Toxicity Category:** [redacted]

2. **Classification:**supplementary

Procedure (Deviations From §81-4):

The test material was instilled in the conjunctival sacs of six pre examined eyes. Observations were at 1, 24, 48, and 72 hrs and on days 7 and 10 according to a scale similar to Draize. On this scale the laboratory states that grade 1 opacity is unremarkable. There is no evaluation of discharge. Although staining was positive in 5/6 eyes at 24 hrs the laboratory reports no opacity, and in the one eye where opacity is observed it seems to be in the area that did not stain. At 48 hrs no staining was done, and 4/5 eyes that had stained at 24 hrs were evaluated as negative for opacity. Yet at 72 hrs two of these still showed positive stain. They were still reported as negative for opacity. At 7 days no staining was observed in any eye, and the laboratory has claimed that the eyes had cleared. Grade 1 redness(4/6) and grade 1 chemosis (1/6) were present on day 7. All eyes are reported negative on day 10.

Results:

Observations	(number "positive"/number tested)							
	Hour	Days						
	1	1	2	3	4	7	10	21
Cornea Opacity	0/6	5/6	1/6?	3/6	-	0/6	0/6	
Iris	4/6	3/6	2/6	2/6	-	0/6	0/6	
Conjunctivae								
Redness	6/6*	6/6*	6/6*	6/6 *	-	4/6*	0/6	
Chemosis	5/6	4/6	4/6*	3/6 *	-	1/6*	0/6	
Discharge	-	-	-	-	-	-	-	

* Grade 1 reaction, unremarkable ? Questionable observation

DATA REVIEW FOR SKIN IRRITATION TESTING (§81-5)

Product Manager:18
MRID No.: 418266-06
Testing Laboratory: Cosmopolitan Safety
Author(s): Geoffrey Robbins
Species: Rabbit, New Zealand White
Age: Adult
Sex:3 M & 3F
Weight:2.0 - 3.5 K
Dosage:0.5 g
Test Material:BMP Technical Powder, 110,000 IU/mg, powder
Quality Assurance (40 CFR §160.12): Included

Reviewer: L. Markarian
Report Date:3/16/88
Report No.:E1824

Summary:

1. **The Primary Irritation Index** = 0.2
2. **Toxicity Category:** IV
3. **Classification:** supplementary

Procedure (Deviations From §81-5):

Test material as weighed per patch was moistened with 0.7 ml of saline to form a paste and applied to the clipped skin of the animals. The site was covered with 1 inch square gauze patch. The trunks of the animals were wrapped in perforated plastic sheeting secured with adhesive tape. At 4 hrs the wrappings were removed ant the site wiped with moist paper towels. The sites were evaluated at 1, 24, 48, 72 hrs according to Draize.

Results:
No greater than grade 1 erythema was observed at any site. All sites appeared normal at 48 hrs.

13

DATA REVIEW FOR SKIN SENSITIZATION TESTING (§81-6)

Product Manager: 18
Reviewer: L. Markarian
MRID No.: 418266-07
Report Date: 4/10/88
Testing Laboratory: Cosmopolitan Safety
Report No.: F1824
Author(s): Geoffrey Robbins
Species: Guinea Pig, Hartley
Weight: 300 - 500 g
Source: Camm Research Lab Animals, Wayne, NJ
Test Material: BMP technical Powder 110,00 IU/mg Lot 28 powder
Positive Control Material: p-phenylenediamine
Quality Assurance (40 CFR §160.12): Included

Method: Modified Buehler

Summary:

1. **This Product is / is not a dermal sensitizer.**

2. **Classification:** Supplementary

Procedure (Deviation From §81-6):

There was no pre test screening for the definition of the induction and elicitation concentrations.
The application for induction and elicitation as a paste made of 500 mg of test material with 0.7 ml of saline (1:1.4 w/v), on the shaved right of the animals. The applications were under 20 X 20 mm Webril Cloth patch under plastic film. The trunks of the animals were wrapped in plastic wrap. Exposure was for 6 hrs. There were three inductions applied once a week for three weeks. Elicitation was two weeks after the last induction at the induction site and at a naive site. The animals were not restrained and there were no naive controls. Reference is given to a positive control test conducted with p-phenylenediamine; however, the results of the test and the time frame in which it was conducted is not included.
Evaluations were at 24 and 48 hours after inductions and challenge according to Draize. The diameter of the reaction is also given.

Results:

All reactions were grade 1 erythema according to Draize as follows:

	I N D U C T I O N			C H A L L E N G E	
	I	II	III	INDUCTION SITE	NAIVE SITE
24 HRS	7/10	8/10	5/10	8/10	7/10
48 HRS	4/10	5/10	5/10	8/10	4/10

The laboratory has concluded that the test material is not a sensitizer.

13

14

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (§ 81-1)

Product Manager:18
MRID No.:419773-01
Testing Facility: Cosmopolitan Safety
Author(s): Geoffrey Robbins
Species: Rat, Sprague Dawley
Age: Young adult
Weight: M 292 - 297 g, F 208 - 212 g
Source: Laboratory Colony
Test Material:BMP 123 (48 LC) Bacillus Thuringiensis var. Kurstaki Lot 5123481 beige liquid
Quality Assurance (40 CFR §160.12):included

Reviewer: L. Markarian
Report Date:6/27/91
Report No.A3203

Conclusion:

1. **The estimated LD_{50} is** > 5g/kg
3. **Tox. Category:** IV **Classification:**Guideline

Procedure (Deviations from §81-1):

Fasted rats were intubated with the test material as received. observations were frequent on the day of intubation and daily thereafter. There were twice daily mortality checks during week days. Body weights were recorded at initiation and on days 7 and 14. Necropsy was performed on all animals.

Results:

Dosage g/kg	(Number Killed/Number Tested)		
	Males	Females	Combined
5.0	0/5	0/5	0/10

Symptoms & Gross Necropsy Findings:
No mortality, signs of toxicity or gross pathology were observed.

DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (§81-3)

Product Manager:18 **Reviewer:** L. Markarian
MRID No.:419773-02 **Report Date:**7/1/91
Testing Laboratory: Cosmopolitan Safety **Report No.:**C3203
Author(s): Geoffrey Robbins
Species: Rat, Sprague Dawley
Weight: M 241 - 268 g, F 201 - 215 g
Source: Laboratory colony
Test Material:BMP 123 (48 LC) Beige liquid
Quality Assurance (40 CFR §160.12): Included

Summary:

1. **The estimated LC_{50} is** >3.59 ml/L

2. **Mean Concentration:**3.59 ml/L

3. **Tox. Category:**III **Classification:**Guideline

Procedure (Deviation From §81-3):

A test and a control group of ten animals each were use to determine the inhalation hazard potential of the test material. The test material was poured through a 20 mu sieve to separate large particles prior to exposure.
Exposure was in a 47.4 liter semicylindrical chamber with an entry port at the top for the aerosol. The exhaust portal was at the opposite side at the bottom. a vacuum pump exhausted the chamber.

The test atmosphere was generated using a DeVibliss model 841 nebulizer and a Gast air pump to supply air(pressure not given). The created aerosol was introduced into the chamber directly from the nebulizer.

Chamber concentrations were measured gravimetrically three times during the exposure. The sampling rate was 1 lpm and duration not more than 10 minutes. Two superimposed filters with activated charcoal inbetween were used for collection of the samples.

Particle size determination was made using a Casella cascade impactor twice during the test.

Chamber air flow, temperature and humidity were monitored and recorded at thirty minute intervals.

Observations were hourly during exposure, after removal from the chamber at 1, 3, and 5 hrs, and daily thereafter. There were twice daily mortality checks during week days.
Necropsy was performed on all animals.

Results:

Chamber Concentration	**TEST**	**CONTROL**
Gravimetric mg/L		
Average	3.59	-------
Range	3.52 - 3.71	
MMAD um		
Average	1.2	
I ± GSD	1.3± 4.3	
II± GSD	1.1± 2.6	
% < 2um		
I	61.3	
II	65.1	
Air Flow lpm	10 constant	10 constant
Temperature range	74 - 86 ^{0}F	76 - 64 ^{0}F
Humidity %	44 - 84	45 - 84
Mortality		
Males	0/5	0/5
Females	0/5	0/5
Combined	0/10	0/10
Signs of Toxicity		
During exposure	Dorsal fur wet	None
Post exposure	None	None
	Normal weight gains at termination	
Necropsy Findings	None	None

Tox Chem No 006402 Current Date 8/4/92
Laboratory: Cosmopolitan Safety Evaluation, Inc., P.O.Box 71, Lafayette, NJ 07848

STUDY	MATERIAL	MRID NO	RESULTS	TOX CAT	CORE GRADE
Oral Toxicity Limit test(Rats) A1824 3/23/88	BMP Technical 110,000 IU/mg Lot 28 Powder	418266-02	LD_{50} > 5.0 g/kg	IV	Minimum
Dermal Toxicity Limit Test(Rabbits) B1824 3/29/88	" " " "	418266-03			Supplementary Upgradeable
Inhalation Toxicity Limit Test (Rats) C1824 4/24/88	" " " "	418266-04			Supplementary
Eye Irritation in Rabbits D1824 3/23/88	" " " "	418266-05	Clear on day 7		Supplementary
Dermal Irritation in Rabbits E1824 3/16/88	" " " "	418266-06			Supplementary Upgradeable
Sensitization in Guinea Pigs F1824 4/10/88	" " " "	418266-07			Supplementary
Oral Toxicity Limit Test(Rats) A3203 6/27/91	BMP 123 (48 LC) Lot 5123481	419773-01	LD_{50}> 5.0 g/kg	IV	Guideline
Inhalation Toxicity Limit Test C3203 7/1/91	" " " "	419773-02	LC_{50} >3.59 mg/L	III	Guideline

17