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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: SACB Review of Data Submitted by Stine Microbial Products to Support the Registration of Blue Circle Inoculant Pseudomonas cepacia) (HED No 1-0959; ID No 063950)

TO: Susan Lewis/Carl Grable (PM-21)
Fungicide-Herbicide Branch
Registration Division (H7505C)

23 SEP 1991

28 SEP 1991

FROM: Cindy Schaffer, Microbiologist CS
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

THROUGH: Reto Engler, Ph.D, Chief
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

DATA REVIEW RECORD

Product Name: Blue Circle Inoculant
Trade Name: SMP-1 PcpWi
Code Name: Pseudomonas cepacia biotype Wisconsin
ID No: 063050-E SMP PCPWI
Caswell No: 714BC
HED Project No: 1-0959
MRID No:

417812-01	SMP PcpWi Product Identity and Disclosure of Ingredients
417812-B2	SMP PcpWi: Analysis of Samples, Certification of Ingredient Limits, and Physical and Chemical Properties
416284-01	Blue Circle Inoculant: Product Identity and Disclosure of Ingredients
417284-02	Blue Circle Inoculant: Analysis of Samples, Certification of Ingredient Limits and Physical and Chemical Properties
415466-01	Acute Oral Toxicity/Pathogenicity-Rat (152A-10)
415466-02	Acute Dermal Toxicity Test-Rabbits (152A-11)
415466-03	Acute Pulmonary Toxicity/Pathogenicity Rat (152A-12)
415466-04	Acute Intravenous Toxicity/Pathogenicity Rat (152A-13)
no # assigned	Hypersensitivity Incidents (152A-15)

ACTION REQUESTED: SACB has been asked to review product analysis and toxicology data in support of the registration of Blue Circle Inoculant SMP-1; Pseudomonas cepacia type Wisconsin strain M36, for use as a seed coating to control fungi that cause damping-off disease (Rhizoctonia, Pythium and Fusarium; lesion (Helicotylenchus ssp.), spiral (Pratylenchus), lance (Heterodera glycines) and root knot (Meloidogyne incognita).

CONCLUSION: SACB can support the application for the registration of Blue Circle Inoculant with the following stipulations:

1. The Taxonomic data for Pseudomonas cepacia is submitted.
2. The registrant identifies the [redacted] found in the TGAI and the methods used for identification.
3. The TGAI must be evaluated for human pathogens, with an accompanying identification scheme, with the verification submitted to the agency.
4. Assessment of storage stability must be documented.
5. The registrant must provide data and methods used to support the certified ingredient limits.

SUMMARY OF DATA SUBMITTED:

Product Identification (151A-10 - 151A-16):

1. The registrant must submit a complete taxonomical evaluation of Pseudomonas cepacia (i.e. general morphology, gram stain identification, flagellar staining data, etc.).
2. The component formulation data needs to be reviewed (The % component by weight is less than 100%).
3. The quality of the TGAI must be assessed. Contaminants such as [redacted] must be identified.
4. The claim of a one year storage stability must be supported by appropriate documentation.
5. Each batch of TGAI must be analyzed for human pathogens (see Subdivision M guidelines 151A-12c). A method for detecting and/or eliminating these pathogens must be in place.
6. Data must be submitted verifying how the certified ingredient limits were established and what the accuracy and precision of the methods used were to set these limits. Generally, 5 or more representative samples are selected for the preliminary analysis of product samples (See subdivision D, Product Chemistry 62-1).

CLASSIFICATION: UNACCEPTABLE

Acute Oral Toxicity/Pathogenicity (152A-10):

Pseudomonas cepacia was neither pathogenic nor infective for rats when orally dosed with approximately 2 x 10^8 CFU per animal of culture/nutrient broth mixture.

CLASSIFICATION: ACCEPTABLE

Acute Dermal Toxicity (152A-11):

P. cepacia [redacted] was not toxic for rats when a single 2 g/animal dose was administered dermally for 15 days.

CLASSIFICATION: ACCEPTABLE

Acute Pulmonary Toxicity/Pathogenicity (152A-12):
Pseudomonas cepacia was neither pathogenic nor infective for rats when dosed intratracheally with 1.9×10^8 CFU of the test material.
CLASSIFICATION: ACCEPTABLE
Acute Intravenous Toxicity/Pathogenicity (152A-13):
Pseudomonas cepacia was not infective, pathogenic or toxic for rats when dosed intravenously with approximately 1.2×10^7 CFU of the test material.
CLASSIFICATION: ACCEPTABLE
Hypersensitivity Incidents:
None reported. The registrant must notify the Agency of hypersensitivity incidents are noted.

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SACB/HED
Secondary Reviewer: J.Thomas McClintock, Ph.D., Microbiologist, SACB/HED

STUDY TYPE:	Product Identity and Disclosure of Ingredients
MRID NO:	416284-01
CASWELL NO:	714BC
TEST MATERIAL:	Blue Circle Inoculant
SYNONYMS:	SMP-1 PcpWi; Pseudomonas cepacia biotype Wisconsin
PROJECT NO:	1-0959
SPONSOR:	Stine Microbial Products, Madison WI
TESTING FACILITY:	Stine Microbial Products, Madison WI
TITLE OF REPORT:	Blue Circle Inoculant: Product Identity and Disclosure of Ingredients
AUTHOR(S):	Janice A. Kimpel, Consultant
STUDY COMPLETED:	20 June, 1990
CONCLUSION:	UNACCEPTABLE: A number of deficiencies were noted including a lack of taxonomic data, defined quality control methods for the end-use product, evaluation of contaminating bacterial species, analysis of human pathogens and methods for evaluating storage stability.

PRODUCT ANALYSIS

151A-10 Product Analysis and Disclosure of Ingredients

Identity: The active ingredient of Blue Circle Inoculant is a bacterial strain identified as Pseudomonas cepacia type Wisconsin.

Confidential Statement of Formula has been submitted. Blue Circle Inoculum contains the active ingredient, Pseudomonas cepacia type Wisconsin (3.8% [redacted] The registrant needs to review the component formulation data. (The % component by weight is less than 100%).

Information on Active Ingredient:

General Taxonomy: was not submitted by registrant.

History: Pseudomonas cepacia type Wisconsin is a biotype of P. cepacia Burkholder, a widely recognized ubiquitous organism with a naturally high diversity. This organism has been isolated from various sources throughout the world, i.e. Australia (Birch 1986), France (Lambert et al. 1987), and the United States (Hagedorn et al. 1987). Biotypes that have been described were initially isolated from clinical specimens, onions (as pathogens), soil and (colonizers of) alder-Frankia nodules.

QUALITY CONTROL PROCEDURE INFORMATION IS NOT INCLUDED

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Active ingredient characterization:

Biochemical/Nutritional Characteristics:

Positive(+) : Reduction of nitrate to nitrite, B-glucosidase, protease, B-galactosidase, assimilation of glucose, arabinose, mannose, mannitol, N-acetyl glucosamine, gluconate, caprate, assimilation of adipate, malate, citrate, phenylacetate.

Negative(-) : Denitrification, indole production, acid production with glucose, arginine dihydrolase, urease, maltose, cytochrome oxidase.

These characteristics are consistent with the Bergey's Manual method of identification for P. cepacia .

Growth as a Function of Temperature: Data was submitted which compared the growth of biotype Wisconsin to other P. cepacia biotypes. P. cepacia biotype Wisconsin grew well at 30 and 37C on nutrient agar and TB-T medium; whereas 2 clinical strains (Hines 1 and 2) and Pseudomonas fluorescens(608) were unable to grow on TB-T medium.

Response of P . cepacia biotype Wisconsin (strain M36) to various antibiotics:

Antibiotic	Minimal Antibiotic Concentration (ug/ml)
Ampicillin	>1000
Carbenicillin	>1000
Chloramphenicol	50
Gentamycin	400
Kanamycin	150
Minocycline	15
Neomycin	350
Polymixin B	>1000
Penicillin B	>1500
Rifampicin	<50
Spectinomycin	>1000
Streptomycin	1500
Tetracycline	100
Trimethoprim	10

The registrant submitted a table showing other P. cepacia variants susceptibility to antibiotics. There was some variability in susceptibility patterns but no clear cut indicator that would distinguish these various biotypes from the biotype Wisconsin strain M36.

Bacteriocin Production and Sensitivity: Bacteriocins are antibiotic compounds that are produced by one bacterium but are active against other bacteria. Cepaciacins are bacteriocins produced by P. cepacia. The registrant submitted data that

clearly shows individual biotypes can be characterized using the criteria of cepaciacin reactivity. Various P. cepacia biotypes are used which are known to produce a cepacian effective against cepacian sensitive or cepacian producer strains. For example: All Wisconsin biotypes can be distinguished from clinical and onion pathogenic P. cepacia isolates by their sensitivity to P. cepacia strains JC2346, B6801 and 18979. Biotype Wisconsin strain M36 is isolated from the other Wisconsin biotypes by its sensitivity to P. cepacia strain 76-16.

Serotyping: The majority of clinical isolates (79 out of 101) were serotyped using eight antisera developed from O (somatic) and H (flagellar) antigens. Ninety percent of the clinical isolates displayed a positive reaction to the O antisera alone. No agglutination was seen when the Wisconsin biotypes were serotyped with the above antigens. This technique and corresponding data indicated that the Wisconsin biotypes are unique and distinct from the clinical isolates.

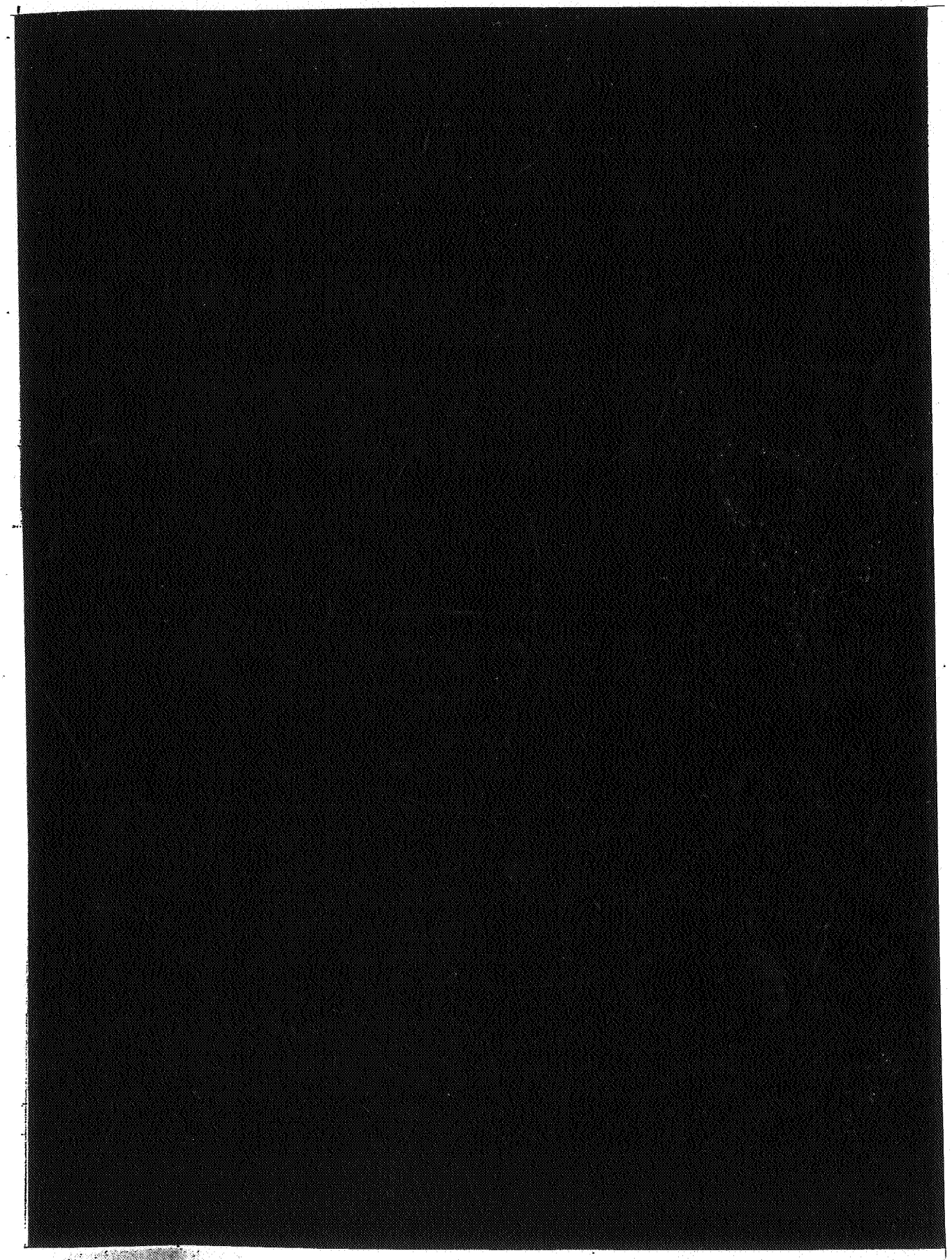
Ribotyping: A ribosomal RNA (rRNA) probe was used by the registrant to differentiate several strains of P. cepacia. When southern blots of EcoR1-digested DNA from several P. cepacia strains were hybridized with a radioactive RRNA probe distinct differences between biotype Wisconsin M36 and other clinical or plant pathogens were observed.

Root Colonization Ability: Various strains of P. cepacia were studied to determine soybean root-colonizing ability. Biotype Wisconsin M36 could colonize 70% of soybean roots while clinical isolates such as B4345 and 527i[2] were only able to colonize 3% of the soybean roots. These findings demonstrate the ability of the biotype Wisconsin M36 to colonize soybean roots while the clinical isolates were unable to colonize to any degree.

Antifungal Activity: P. cepacia isolates were tested against the following plant pathogenic fungi for zone inhibition: Fusarium moliniforme, Pythium ultimum, Phytopthora megasperma, Rhizoctonia solani, and Sclerotinia sclerotiorum. Although other plant pathogenic or clinical strains of P. cepacia were able to show zonal inhibition to select fungi; only biotype Wisconsin M36 demonstrated inhibition to all of these fungi.

[redacted]

INERT INGREDIENT INFORMATION IS NOT INCLUDED



QUALITY CONTROL PROCEDURE INFORMATION IS NOT INCLUDED

INERT INGREDIENT INFORMATION IS NOT INCLUDED QUALITY CONTROL PROCEDURE INFORMATION IS NOT INCLUDED

[redacted]

Physical/Chemical Properties (151A-16):

	Technical Grade A.I.	End Use Product
Color	pale yellow	dark brown
Physical State	liquid suspension	solid
Odor	typical culture broth	earthy, grain like
Specific Gravity	N/A	0.6
pH	5.5	6.0 to 7.0
Stability	not stable to sunlight, stable at neutral pH	not stable to sunlight stable at neutral pH
Temp. Stability	10-75 F	10-75 F
Storage Stability	One month on agar plates; indefinitely as lyophilized cultures or glycerol stock (-90 C)	one year in a cool, dry place
Corrosion	N/A	Bags should be kept dry in a cool place and out of direct sunlight

DISCUSSION

Although the registrant submitted extensive data which ensures biotype specificity, the general taxonomic data (i.e. general morphology, gram stain identification, flagellar staining data etc.) on the genus Pseudomonas was not included in the package for registration. This information is necessary to support the

taxonomic criteria. Along with this, the following deficiencies were noted:

1. The registrant needs to review the component formulation data (The % component by weight is less than 100%).
2. A defined method for evaluating the quality of the TGAI needs to be addressed. This must include methods for identifying and evaluating contaminants [redacted].
3. Data must be submitted to support the claim of a 1 year storage stability.
4. Each batch of the TGAI must be analyzed for human pathogens (see Subdivision M guidelines 151A-12c). A method for detecting and/or eliminating these pathogens must be in place.
5. The registrant is required to submit data explaining how the certified ingredient limits were established and what the accuracy and precision of the methods used were to set these limits. Generally, 5 or more representative samples are selected for the preliminary analysis of product samples. (See Subdivision D, Product Chemistry 62-1).

QUALITY CONTROL PROCEDURE INFORMATION IS NOT INCLUDED

INERT INGREDIENT INFORMATION IS NOT INCLUDED

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SACB/HED
Secondary Reviewer: J.Thomas McClintock, Ph.D., Microbiologist, SACB/HED

STUDY TYPE:	Acute Oral Toxicity/Pathogenicity-Rat(152A-10)
MRID NO:	415466-01
CASWELL NO:	714 6C
TEST MATERIAL:	Pseudomonas cepacia
SYNONYMS:	SMP-1
PROJECT NO:	G-7247.222
SPONSOR:	Stine Microbial Products, Madison WI
TESTING FACILITY:	Microbiological Associates Inc. Bethesda MD
TITLE OF REPORT:	Acute Oral Toxicity/Pathogenicity Study of SMP-1 in Rats
AUTHORS(S):	Raymond M. David Ph.D, D.A.B.T.
STUDY COMPLETED:	21 February 1990
CONCLUSION:	Pseudomonas cepacia was neither pathogenic nor infective for rats when dosed orally with approximately 2 x 10^8 CFU's of culture/nutrient broth mixture.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN

Test Material: The microbial pesticide control agent is Pseudomonas cepacia culture in nutrient broth. The potency was calculated to be 2.85 x 10^9 colony forming units (CFU)/ml. Each test animal received a 0.1 ml dose (approximately 1.4 x 10^8 cfu) by oral gavage.

Test Animals: Nineteen male and nineteen female Sprague Dawley rats, approximately 56 days old, were obtained from Harlan Sprague Dawley Inc. Frederick MD. The male rats weighed between 172g and 250g and females weights ranged from 141g to 176g at the beginning of the study.

Methods: The animals were assigned as follows: The untreated animal group was comprised of four male and four female rats; the treatment group contained fifteen female and fifteen male rats. The rats were randomly weighed before initial dosing and weekly thereafter. Treated animals were observed for signs of toxicity at 1 and 4 hours after dosing and on a daily basis thereafter. Three rats of each sex in the treatment group were sacrificed by CO_2 asphyxiation at days 1, 3, 7, 14 and 23 days after dosing. Four rats per sex from the non-treated group were sacrificed on day 23, the last day of the study. The animals were examined by necropsy for any macroscopic abnormalities. The number of viable CFU observed in the broth/culture mixture and rat organs

were determined by plating 100 µl aliquots of homogenized tissue samples in duplicate on (what SACB presumes to be) TBT media + 5 mg/l Kanamycin plates. If necessary, samples were diluted in phosphate buffered saline in an attempt to obtain a reasonable number to count (< 100 CFU/plate). The plates were incubated at 35.0 ± 0.1 C for at least 40 hours. Infectivity determinations were based on colonies per plate and dilution of tissue required for preparation. Therefore, one colony per plate was equal to 30 colonies per gram of tissue.

II. RESULTS

A. Body Weights:

The mean body weight gain for male treated rats on day 8 of the study was 18.4% ± 10.96%; while the mean weight gain during the same period for non-treated male rats was 12.3% ± 4.11%. The treated females showed a 19.4% ± 3.17% increase in body weight during the first 8 days while the non-treated females experienced 8.9% ± 5.03% body weight gain. By the end of the study, the treated males and females gained an average of 40.5% and 35.6% respectively, while the non-treated male and female rats gained 24.9% and 19.3% of their body weight. Although the treated rats gained more weight than the controls, this difference was not reported to be significant.

B. Clinical observations:

One treated male rat died on day 2. Red or brownish red secretions from the eyes were seen in one treated female on days 4, 6 and 7; and in one male control rat on days 6 and 8.

C. Necropsy observations:

Treated Rats--Day 2:		1 male exhibited clotted blood in the chest cavity
	Day 15:	1 male had severe fibrinous adhesions between the pleural surfaces of the thoracic cavity, lungs and pericardial sac.
	Day 23:	2 females demonstrated grey colorings on one or both lungs.

No abnormalities were observed in any of the control rats upon necropsy.

D. Microbial clearance/infectivity:

Clearance and infectivity were evaluated in the brain, blood, lymph nodes, kidney, liver, spleen, lungs and feces. The submitted data (see below) demonstrated that isolated incidences of the organism were detected in the lymph nodes, spleen and lungs. In all instances, the test material was

cleared from these organs by day 23..The test material, P. cepacia was also isolated from the feces day 2 through day 4; however clearance was demonstrated by day 8.

ORGAN	DAY	# rats*	CFU/GM OF TISSUE
Lymph nodes	2	1/5	90
	4	1/6	30
Spleen	2	1/5	30
	4	1/6	30
Lungs	4	1/6	480
	15	1/6	600
Feces	2	4/5	600
			1.80×10^3
			5.94×10^4
			3.18×10^4
	3	1/6	1.20×10^3
	4	2/6	600
			1.20×10^3

* # of (+) treated rats/ total # of treated rats

III. SACB DISCUSSION:

Treated male and female rats showed an overall higher percentage weight gain compared to the control rats (males: 40.5% vs 24.9%; females: 35.6% vs 19.3%) during the 23 day study. This difference was not reported as being significant. There were few clinical observations noted. One treated rat died on the 2nd day of the study; apparantly due to dosage error (clotted blood was found in the chest cavity); and two rats exhibited a reddish/brown occular discharge (1 treated female; days 4, 6 and 7 and 1 non-treated male; days 6 and 8). The necropsy observations revealed one male, (day 15) with severe fibrinous adhesions in the thoracic cavity; and two females (day 23) with a grey coloring on the lungs. The organism was recovered from the lymph nodes, spleen and lungs in a few isolated instances. The test animal showed a distinct clearance pattern from the above organs by day 23 and from the feces by day 8. Based on the submitted data, when administered orally, the test material was not infectious, pathogenic or toxic to rats.

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SACB/HED
Secondary Reviewer: J.Thomas McClintock, Ph.D., Microbiologist, SACB/HED

STUDY TYPE:	Acute Dermal Toxicity Test-Rabbits(152A-11)
MRID NO:	415466-02
CASWELL NO:	714BC
TEST MATERIAL:	Pseudomonas cepacia [redacted]
SYNONYMS:	SMP-1
PROJECT NO:	G-7254.232
SPONSOR:	Stine Microbial Products, Madison WI
TESTING FACILITY:	Microbiological Associates Inc. Bethesda MD
TITLE OF REPORT:	Acute Dermal Toxicity Test of SMP-1 [redacted] (P. cepacia) in Rabbits
AUTHOR(S):	Raymond M. David Ph.D, D.A.B.T.
STUDY COMPLETED:	21 February 1990
CONCLUSION:	SMP-1 [redacted] was not toxic for rats when a single 2 g/animal dose was administered dermally.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN

Test Material: The microbial pesticide control agent is Pseudomonas cepacia [redacted] The purity and stability of the test article was not determined. The potency was determined by plating out dilutions in triplicate on TBT plates and incubating them for 40 to 45 hours at 35C.The final concentration was determined to be 2.4×10^9 colony forming units (CFU)/gm.

Test Animals: Following a 9 day quarantine period, five male and five female New Zealand albino rabbits, approximately 12 to 18 weeks of age, were obtained from Buckshire Corporation, Perkasie PA. The rabbits weighed between 2.4 and 2.7 kg at dosing.

Methods: Approximately 24 hours prior to testing, no less than 10% of the trunk fur (about $240cm^2$ of dorsal body surface) was clipped. The saline moistened [redacted] preparation was applied to the test area followed by a covering of gauze and adhesive tape. Next, each rabbits' entire midsection was securely wrapped with a cloth toweling followed by adhesive tape. Approximately 24 hours following application, all wrappings were removed from the animal and the skin was wiped clean of excess test material using saline moistened paper towels. The rabbits were observed for clinical signs of toxicity once prior to dosing, at one and three hours post dosing, and once daily during the 14 day observation period.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Body weights were obtained on day 1, day 8 and day 15 prior to necropsy. The animals were sacrificed on the last day of the study. A gross necropsy, including examination of the external test site surface, all orifices, cranial cavity, carcass, external surfaces of the brain, one cross section of spinal cord, nasal cavity and paranasal sinuses, the thoracic, abdominal and pelvic cavities and their viscera, and the cervical tissues and organs was performed on all test animals.

II. RESULTS

A. Body Weights:
The body weight gain of the animals were not affected by the test substance (see below).
Mean Body Weights(kg):

Day#	Male	Female
Day 1	2.5 ± 0.1	2.6 ± 0.1
Day 8	2.6 ± 0.1	2.8 ± 0.1
Day 15	2.7 ± 0.1	2.9 ± 0.1

B. Clinical observations:
No clinical signs of toxicity were noted.

C. Necropsy observations:
One male rabbits lungs were partially inflated and discolored grey. No abnormalities were observed in any other rabbit upon necropsy.

III. SACB DISCUSSION:

The body weights were not affected by the test organism. No clinical signs of toxicity were noted. One male rabbit experienced grey, discolored and partially inflated lungs. No other gross lesions were noted. Based on the submitted data, when administered dermally, the test material was not toxic to rats.

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SACB/HED
Secondary Reviewer: J.Thomas McClintock, Ph.D, Microbiologist, SACB/HED

STUDY TYPE:	Acute Pulmonary Toxicity/Pathogenicity-Rat (152A-12)
MRID NO:	415466-03
CASWELL NO:	714BC
TEST MATERIAL:	Pseudomonas cepacia
SYNONYMS:	SMP-1
PROJECT NO:	G-7247.225
SPONSOR:	Stine Microbial Products, Madison WI
TESTING FACILITY:	Microbiological Associates Inc. Bethesda MD
TITLE OF REPORT:	Acute Pulmonary Toxicity/Pathogenicity Study SMP-1 in Rats.
AUTHOR(S):	Raymond M. David Ph.D, D.A.B.T.
STUDY COMPLETED:	21 February, 1990
CONCLUSION:	Pseudomonas cepacia was neither pathogenic nor infective for rats when dosed intratracheally with approximately 1.9×10^8 CFU of the test material.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN

Test Material: The microbial pesticide control agent is Pseudomonas cepacia in sterile saline. The potency of the dosing solution was calculated to be 4.75×10^9 colony forming units (CFU)/ml. Each test animal received a 0.04 ml dose (approximately 1.9×10^8 CFU) intratracheally.

Test Animals: Twenty-eight male and twenty-eight female Sprague-Dawley rats, approximately 64 days old, were obtained from Harlan Sprague Dawley Inc. Fredrick MD. The male rat's weight ranged from 254.1 gm to 308.3 gm and the female's weight ranged from 164.0 gm to 229.8 gm at the beginning of the study.

Methods: The animals were assigned to two groups as follows: The untreated animal group was comprised of four male and four female rats; the treatment group contained twenty-four female and twenty-four male rats. The rats were randomly weighed before initial dosing and weekly thereafter. Treated animals were observed for signs of toxicity at 1 and 4 hours after dosing and on a daily basis thereafter. Three rats of each sex in the treatment group were sacrificed for infectivity determinations by CO_2 asphyxiation at 1, 3, 7, 14 and 22 days after dosing. Four rats per sex from the non-treated group were sacrificed for gross necropsy on day 23, the last day of the study. The

animals were examined by necropsy for any macroscopic abnormalities. Lungs from three animals per sex from the treated group were collected immediately after dosing (within 1 hour) to evaluate clearance of the test microbe. A preliminary study was performed to determine the sensitivity of P. cepacia to Kanamycin. The tissue samples from control animals were taken at necropsy, known quantities of the test microbe added, tissue homogenates plated, then incubated on antibiotic supplemented medium at 35C for at least 40 hours. The test microbe recovery was determined to be 72.1%. The number of viable CFUs observed in the initial dose and rat organs were determined by plating 100 µl aliquots of homogenized tissue samples in duplicate on (what SACB presumes to be) TBT media (containing 20mg/l Tetracycline) + 5 mg/l Kanamycin plates. If necessary, samples were diluted in phosphate buffered saline in an attempt to obtain a reasonable number to count. (< 100 CFU /plate). The plates were incubated at 35.0 ± 0.1 C for at least 40 hours. Infectivity determinations were based on colonies per plate and dilution of tissue required for preparation. Therefore, one colony per plate was equal to 30 colonies per gram of tissue.

II. RESULTS

A. Body Weights:

The treated male rats showed an overall increase in body weight of 15.2% while the male control weight increased 17%. The treated female rats showed an overall increase of 11.2%; while the non-treated female body weight increased by 8.4%. These values were not considered significant by the registrant.

B. Clinical observations:

Two treated male and two treated female rats showed signs of a ruffled coat and rapid shallow breathing on day 1. One treated male exhibited a red secretion from the right eye on day 2; and one treated female had red and brown secretions from both eyes from days 3 to 9 and 11 to 15.

C. Microbial clearance/infectivity:

Clearance and infectivity were evaluated in the brain, blood, lymph nodes, kidney, liver, spleen, lungs and caecum. The submitted data (see below) demonstrated that isolated incidences of the organism were detected in the brain and spleen on day 4. In these instances, the test material was cleared from these organs by day 8. The test material, P. cepacia, was also isolated from the lungs from day 2 through day 15; however clearance was demonstrated by day 22.

ORGAN	DAY	#RATS*	MEAN CFU/GM OF TISSUE
Brain	4	1/6	5
Spleen	4	1/6	5
Lungs	2	6/6	1.8×10^6
	4	6/6	2.34×10^5
	8	5/6	168
	15	1/6	5

*# of (+) treated rats/# treated rats

D. Necropsy observations:

Treated Rats:

Day 2 — 1 Male: Exhibited brown discoloration (2 to 5 mm diameter) on the lungs

Day 4 — 3 Males: Slight to grey discoloration of one or both lungs. Raised lungs with grey spots.

2 Females: Slight to grey discoloration on lungs

1 Female: Grey spot on the back near base of liver

Day 8 — 1 Male: Lungs were brownish in color

1 Male: White spots on right lung

2 Females: Brown color on lungs

Control Rats: No abnormalities were observed upon necropsy.

III. SACB DISCUSSION

The treated and control male rats exhibited an average body weight gain of 15.2% and 17% respectively. The mean body weight of treated and control female rats increased by an average of 11.2% and 8.4% respectively. These weight gains were not noted by the registrant as being significantly different. Four treated rats (2 male, 2 female) showed signs of rapid shallow breathing and a ruffled coat on day 1. Red eye secretions were also noted in one treated male on day 2 and one treated female on days 3 through 9 and 11 through 15. The organism was recovered from the brain and spleen in a few isolated instances and from the lungs from days 2 through 15. The test animal showed a distinct clearance pattern of the test material from the brain and spleen by day 8 and from the lungs by day 23. The necropsy observations revealed 6 males and 4 females with a grey or brown discoloration of the lungs through day 8 and one female exhibited a grey spot on the back near the base of the liver. Based on the submitted data, when administered intratracheally, the test material was not infectious, pathogenic nor toxic to rats.

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SACB/HED
Secondary Reviewer: J.Thomas McClintock, Ph.D., Microbiologist, SACB/HED

STUDY TYPE:	Acute Intravenous Toxicity/Pathogenicity-Rat (152A-13)
MRID NO:	415466-04
CASWELL NO:	714BC
TEST MATERIAL:	Pseudomonas cepacia
SYNONYMS:	SMP-1
PROJECT NO:	G-7247.224
SPONSOR:	Stine Microbial Products, Madison WI
TESTING FACILITY:	Microbiological Associates Inc. Bethesda MD
TITLE OF REPORT:	Acute Intravenous Toxicity/Pathogenicity Study of SMP-1 in Rats
AUTHORS(S):	Raymond M. David Ph.D, D.A.B.T.
STUDY COMPLETED:	21 February 1990
CONCLUSION:	Pseudomonas cepacia was not infective, pathogenic or toxic for rats when dosed intravenously with approximately 1.2×10^7 CFU of the test material.
CLASSIFICATION:	Acceptable

I. STUDY DESIGN

Test Material: The microbial pesticide control agent is Pseudomonas cepacia in sterile saline. The potency of the dosing solution was calculated to be 6.0×10^7 colony forming units (CFU)/ml. Each test animal received a 0.2 ml dose (approximately 1.2×10^7 cfu) intravenously.

Test Animals: Twenty-six male and twenty-six female Sprague-Dawley rats, approximately 58 days old, were obtained from Harlan Sprague Dawley Inc. Frederick MD. The male rats weight ranged from 221.0 gm to 289.9 gm and the females weight range from 146.3 gm to 206.6 gm at the beginning of the study.

Methods: The animals were assigned to two groups as follows: The untreated animal group was comprised of two male and two female rats; the treatment group contained twenty-four female and twenty-four male rats. The rats were randomly weighed before initial dosing and weekly thereafter. Treated animals were observed for signs of toxicity at 1 and 4 hours after dosing and on a daily basis thereafter. Three rats of each sex in the treatment group were sacrificed by CO_2 asphyxiation for infectivity determinations at days 1, 3, 7, 14 and 23 days after dosing. Four rats per

sex from the non-treated group were sacrificed for gross necropsy on day 22, the last day of the study. All sacrificed animals were examined by necropsy for any macroscopic abnormalities. Blood from three animals per sex from the treated group were collected immediately after dosing (within 1 hour) to evaluate clearance of the test microbe. A preliminary study was performed to determine the sensitivity of P. cepacia to Kanamycin. The tissue samples from control animals were taken at necropsy, known quantities of the test microbe added, tissue homogenates plated, then incubated on antibiotic supplemented medium at 35 C for at least 40 hours. The test microbe recovery was determined to be 72.1%. The number of viable CFUs observed in the initial dose and in rat organs following dosing were determined by plating 100 µl aliquots of homogenized tissue samples in duplicate on (what SACB presumes to be) TBT media (containing 20mg/l tetracycline) + 5 mg/l Kanamycin plates. If necessary, samples were diluted in phosphate buffered saline in an attempt to obtain a reasonable number to count (<100 CFU/plate). The plates were incubated at 35.0 ± 0.1 C for at least 40 hours. Infectivity determinations were based on colonies per plate and dilution of tissue required for preparation. Therefore, one colony per plate was equal to 30 colonies per gram of tissue.

II. RESULTS

A. Body Weights:

The treated male rats showed an overall increase in body weight of 22.5% while the male control weight increased 15.3%. The treated female rats showed an overall increase of 21.5%; while the body weight of the non-treated females increased by 13.5%. The registrant did not consider these values to be significantly different.

B. Clinical observations:

One treated male exhibited a red secretion from the right eye on day 2; and one male control had brownish red secretions from both eyes from days 4 through 8 and 20 through 22.

C. Microbial clearance/infectivity:

Clearance and infectivity were evaluated in the brain, blood, lymph nodes, kidney, liver, spleen, lungs and caecum. The submitted data (see below) demonstrated that the organism was detected in the kidney, spleen, liver and lungs through day 22. The test material was seen in the blood at 1 hour post dosing (mean = 5.64×10^3 CFU) and cleared from the blood by day 2. P. cepacia was also isolated from the lungs from day 2 through day 8 and again on day 15; however a clearance pattern of the test organism was demonstrated throughout the course of the study.

ORGAN	DAY	# rats*	MEAN CFU/GM OF TISSUE
Kidney	2	2/6	10
	4	2/6	10
	8	1/6	60
	22	1/6	5
Spleen	2	2/6	8.50×10^3
	4	6/6	690
	8	2/6	15
	22	1/6	5
Liver	2	3/6	5.94×10^3
	4	5/6	165
	8	1/6	10
Lungs	2	1/6	135
	4	4/6	55
	8	2/6	10
	22	2/6	15

* # of (+) treated rats/ total # of treated rats

D. Necropsy Evaluations:

Treated Rats--Day 8: 1 Male: exhibited grayish discoloration on left lung

Day 22: 1 Male: brownish color on back of both lungs

Control Rats-- No abnormalities were observed upon necropsy.

III. SACB DISCUSSION:

The treated and control male rats exhibited an average body weight gain of 22.5% and 15.3% respectively. The body weight of the treated and control female rats increased an average of 21.5% and 13.5% respectively. These weight gains were not noted by the registrant as being significantly different. One treated male rat showed signs of a red discharge from the right eye on day 2. Brownish red eye secretions were also noted in one control male on days 4 through 8 and days 20 through 22. The test organism was recovered (30 to 60 cfu/gm tissue) from the kidney, spleen, liver and lungs throughout the study. However, by the end of the study, the test animal showed a distinct clearance pattern. The necropsy observations revealed 2 males, one on day 8 and one on day 22, with a grey or brown discoloration of the lungs. Based on the submitted data, when administered intravenously, the test material was not infectious, pathogenic or toxic to rats.