US Environmental Protection Agency
Office of Pesticide Programs

BIOPESTICIDES REGISTRATION ACTION DOCUMENT

TRYPSIN MODULATING OOSTATIC FACTOR (TMOF)

(PC Code 105403)

BIOPESTICIDE REGISTRATION ACTION DOCUMENT

TRYPSIN MODULATING OOSTATIC FACTOR (TMOF)

(PC Code 105403)

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division
Trypsin Modulation Oostatic Factor (TMOF)
(PC Code 105403)

TABLE OF CONTENTS

BIOPESTICIDES REGISTRATION ACTION DOCUMENT TEAM

Office of Pesticide Programs:

Biopesticides and Pollution Prevention Division

Microbial Pesticides Branch

Health Effects

John Kough	Biologist, Senior Scientist
Roy Sjoblad	Biochemist, Senior Scientist
Carl Etsitty	Microbiologist
Joel Gagliardi	Microbial Ecologist

Ecological Effects

Zigfridas Vaituzis	Microbiologist, Senior Scientist
Robyn Rose	Entomologist

Regulations

Phil Hutton	Entomologist, Associate Division Director
Dennis Szuhay	Botanist, Branch Chief
Jim Downing	Environmental Protection Specialist, Acting Team Leader
Alan Reynolds	Entomologist, Regulatory Action Leader

I. EXECUTIVE SUMMARY

A. IDENTITY

The active ingredient, trypsin modulating oostatic factor (TMOF), is a 10-amino acid protein (decapeptide) whose genetic coding was isolated from a mosquito and engineered into *Pichia pastoris* yeast. The manufacturing-use product (Technical Trypsin Modulating Oostatic Factor) contains 1.1% TMOF and is intended to be manufactured into end products for control of mosquito larvae. An end-use product has not been proposed at this time. The mode of action of TMOF is hormonal disruption of transcription and translation of trypsin, resulting in reduced digestion of mosquito diet ultimately leading to starvation of mosquito larvae.

The product chemistry data submitted by the registrant satisfies the requirement for product identity except for storage stability.

B. USE/USAGE

The manufacturing-use product, Technical TMOF, will be used for incorporation into end-use products intended for water applications to control mosquito larvae. An end-use product has not been proposed for registration at this time.

C. RISK ASSESSMENT

No unreasonable adverse effects on humans and the environment are anticipated from aggregate exposure to TMOF.

1. Human Health Risk Assessment

a. Toxicological Endpoints

No toxicological endpoints were identified. Submitted data for TMOF Technical indicate Toxicity Category III for acute oral toxicity and dermal irritation and Toxicity Category IV for acute dermal toxicity and acute inhalation toxicity. Submitted mutagenicity data revealed no mutagenic activity associated with TMOF. Requests for data waivers have been granted for primary eye irritation, dermal sensitization, 90-day studies (feeding, dermal, and inhalation), teratogenicity, and immunotoxicity. The Signal Word, Precautionary Statements, First Aid Statement and other label statements are sufficient to protect from any adverse reactions that may occur from exposure to TMOF.

b. Human Exposure

The Agency has considered the cumulative effects of TMOF and other substances in relation to a common mechanism of toxicity. These considerations include the possible cumulative effects of such residues on infants and children. There is no indication of mammalian toxicity at the maximum doses tested, of this technical product containing TMOF.

The pesticide product, Technical TMOF, is for manufacturing use only. The potential for exposure to the pesticide exists only for manufacturers. Because of low acute mammalian toxicity, worker exposure data on TMOF are not required. The "caution" statement and hazard and first aid statements on the label are sufficient to protect from any adverse reactions that may occur from exposure to TMOF. In addition, the label will bear a dust mask statement to mitigate any potential risk from the inhalation of microbial proteins.

End use products formulated from Technical TMOF may be applied directly to water for control of mosquito larvae. However, such products will be restricted from finished, treated drinking water sources. Each end product will be evaluated individually to determine any risk or labeling issues.

c. Risk Assessment

The Biopesticides and Pollution Prevention Division (BPPD) has not identified any subchronic, chronic, immune, endocrine, or nondietary exposure issues that may affect children or the general U.S. population. Risk to users is mitigated as long as the product is used according to label directions. No toxicological endpoints have been identified, and there is limited exposure to this product since it is for manufacturing use. The Agency has considered TMOF in light of the relevant safety factors in the Food Quality Protection Act (FQPA) of 1996 and under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and has determined that there will be no unreasonable adverse effects from its use as a manufacturing use product to formulate end products for control of mosquito larvae.

2. Ecological Risk Assessment

a. Ecological Toxicity Endpoints

A submitted avian oral toxicity study showed no toxicity or adverse effects from TMOF. Five aquatic non-target toxicity tests were submitted (covering four indicator species), all showing no toxicity or adverse effects from TMOF. A separate aquatic microcosm study showed that a *freshwater environment was not a conducive environment for Pichia pastoris yeast cells containing TMOF and did not support reproduction of the yeast cells.* All other ecological data requirements were waived.

b. Ecological Exposure

The proposed product, Technical TMOF, is a manufacturing use product that is not to be directly applied to the environment. End products formulated from TMOF will be applied directly to water for control of mosquito larvae. Environmental fate was addressed in a submitted aquatic microcosm study which showed that yeast cells containing TMOF do not reproduce in simulated freshwater environments. Ground water data are not available for TMOF. Exposure assessments on this type of product (biochemical pesticide) are not performed unless significant human health or ecological effects issues arise in the Tier I studies for either of these disciplines

(40 CFR §158.690 (c)) and (d)). Since Tier II studies were not triggered, there is no requirement for environmental fate data.

c. Risk Assessment

The submitted ecological data and waivers support a conclusion of reasonable certainty that no incremental hazards to non-target organisms or to the environment are expected as a result of the intended use of TMOF.

D. DATA GAPS / LABELING RESTRICTIONS

There are no data gaps.

II. OVERVIEW

A. ACTIVE INGREDIENT OVERVIEW

Chemical Name: Trypsin Modulation Oostatic Factor (TMOF), as expressed in *Pichia pastoris*

Chemical Formula: H_2N-Tyr-Asp-Pro-Ala-$(Pro)_6$-COOH

Chemical Family: Protein (decapeptide insect hormone)

Trade and Other Names: Skeetercide

CAS Registry Number: Not available

OPP Chemical Code: 105403

Basic Manufacturer: Insect Biotechnology, Inc.
P.O. Box 2311
Chapel Hill, NC 27515

B. USE PROFILE

The following is information on the proposed uses with an overview of use sites and application methods.

Type of Pesticide: Biochemical insecticide

Method and Rates of Application: End-use products formulated from the manufacturing-use product (Technical TMOF) will be applied to water for control of mosquito larvae.

Target Pests: Mosquito larvae

Formulation Types: Solid powder

Use Sites: Manufacturing use only. For incorporation into end-use products as a mosquito larvicide.

Use Practice Limitations: "For Manufacturing Use Only"
"For Use in Manufacturing or Formulating Registered Pesticide Products"

C. ESTIMATED USAGE

None used yet since this will be the first registered product.

D. DATA REQUIREMENTS

The data requirements for granting this registration under Section 3(c)(5) of FIFRA have been reviewed by the Biopesticides and Pollution Prevention Division (BPPD). The mammalian toxicology and ecological effects data requirements for Technical TMOF have been fulfilled. Product chemistry data requirements are adequately satisfied except for storage stability. Based on submitted information, the Agency foresees no unreasonable adverse effects to human health and the environment from the use of Technical TMOF, and recommends a conditional registration.

E. REGULATORY HISTORY

A Notice in the Federal Register on October 9, 2002 (Volume 67, Number 196, pages 62695-62697) announced receipt of an application to register the pesticide product Technical Trypsin Modulation Oostatic Factor (EPA Reg. No. 74411-R) by Insect Biotechnology, Inc. with a 30-day comment period. The product contains a new active ingredient, trypsin modulating oostatic factor (TMOF). No comments were received as a result of this publication.

F. CLASSIFICATION

Although expressed in a microbe (*P. pastoris* yeast), TMOF is classified as a biochemical pesticide. This is because the yeast is inactivated (heat-killed) during production and will not be viable in the environment.

G. FOOD CLEARANCES/TOLERANCES

The proposed pesticide is a manufacturing-use product (Technical TMOF). There is no end use product currently proposed. Therefore, a tolerance or exemption from tolerance was not established concurrent with this action. In addition, end-use products formulated from Technical TMOF will be used for mosquito control in aquatic environments, a non-food use. However, should the registrant seek any food use sites for the end-use product, the requirement of a tolerance must be addressed.

III. SCIENCE ASSESSMENT

A. PHYSICAL/CHEMICAL PROPERTIES ASSESSMENT

All product chemistry data requirements for Technical TMOF are satisfied for the manufacturing-use product (referred to as TMOF in this section) except for storage stability.

1. Product Identity and Mode of Action

a. Product Identity:

The product chemistry data submitted by the registrant satisfies the requirement for product identity (BPPD Reviews dated September 17, 2003 and January 27, 2004). TMOF *Pichia pastoris* (EPA Reg. No. 74411-R) is a TGAI for manufacturing use only; a decapeptide insect hormone. TMOF is used to down-regulate digestive trypsin-like protease translation and transcription after passing into the hemolymph from the midgut, causing larvae to succumb to starvation. The active ingredient, TMOF (CAS No. not available, PC code 105403), is cloned into the yeast *Pichia pastoris* and expressed after induction by methanol. Levels of TMOF in dried TMOF *Pichia pastoris* amount to 2±1 % (MRID 455244-07), 0.2-2.2 % (MRID 457367-03), or ≤ 2 % (MRID 457367-04) of dried *Pichia pastoris* yeast-cell mass. The chemical name of the active ingredient is H_2N-Tyr-Asp-Pro-Ala-$(Pro)_6$-COOH (YDPAPPPPPP). The host range of TMOF *Pichia pastoris* is presented in MRID 455244-07 and 457367-01, and below as Table 1a; exposure of the non-target insects reportedly affected by TMOF (by Trypsin-like enzyme biosynthesis reduction) is not expected since end use products formulated with the TGAI are intended for application to water.

Although TMOF is cloned into *Pichia pastoris* yeast, a microbe, it is classified as a biochemical pesticide. This is because the yeast is heat-killed during production so that no viable (live) cells are present in the final product. The inactivation process is confirmed with appropriate quality control tests (detailed in EPA letter to IBI, dated 3/26/02 and MRID 455244-03).

TABLE 1a: Host range and effects of TMOF.

Family	Genus and species	Common name(s) reported	Exposure	Trypsin-like enzyme biosynthesis effects
DIPTERA	*Aedes aegypti*	Mosquito (target pest)	Feeding	Yes
	Aedes albopticus	Mosquito (target pest)	Feeding	Yes
	Aedes taneorhynchus	Mosquito (target pest)	Feeding	Yes
	Culex quinquefasciatus	Mosquito (target pest)	Feeding	Yes
	Culex nigripalpus	Mosquito (target pest)	Feeding	Yes
	Anopheles quadrimaculatus	Mosquito (target pest)	Feeding	Yes
	Anopheles albimanus	Mosquito (target pest)	Injection	Yes
	Lutzomyia anthophora	Sand fly (Adult female)	Injection	Yes

Family	Genus and species	Common name(s) reported	Exposure	Trypsin-like enzyme biosynthesis effects
	Stomoxys calcitrans	Stable fly (Adult female)	Injection	Yes
	Musca domestica	House fly (Adult female)	Injection	Yes
	Neobellieria bullata	Fleshfly (Larvae and adult female)	Injection	None
			Feeding	
	Culicoides variipennis	No-See-'Ems	Injection	Yes
	Ctenocephalides felis (bouche)	Cat flea (Adult female)	Injection	Yes
LEPIDOPTERA	*Heliothis virescens*	Tobacco budworm (Larvae)	Injection	Reduced gut trypsin
			Feeding	None
	Spodoptera litura	Egyptian cotton leafworm armyworm, cotton worm, common cutworm (Larvae)	Feeding	None
	Agrotis segetum	Black cutworm, turnip moth (Larvae)	Unknown	Growth inhibition
	Plutella xylostella	Diamondback moth (Larvae)	Feeding	None
	Cydia pomonella	Codling moth (Larvae)	Feeding	None
	Lymantria dispar	Gypsy moth	Feeding	Growth retardation & mortality
	Helicoverpa zea	Corn Earworm	Feeding	Growth retardation & limited mortality
	Spodoptera frugiperda	Fall Armyworm	Unknown	Some Retarded growth
	Spodoptera exigue	Beet Armyworm	Unknown	Some Retarded growth
	Leptinotarsa decemlineata	Colorado Potato Beetle	Unknown	Some Retarded growth
	Manduca sexta	Tobacco hornworm (Larvae)	Feeding	None
	Tricoplusia ni	Cabbage looper (Larvae)	Feeding	None
COLEOPTERA	*Anthonomus grandis*	Cotton boll weevil (Larvae)	Feeding	Yes
	Diaprepes abbreviatus	Citrus weevil (Larvae)	Feeding	Yes
HEMIPTERA	*Bemisia spp.*	White Fly	Unknown	Very weak activity
	Trialeurodes vaporarium	Greenhouse Whitefly	Feeding	Yes
HYMENOPTERA	*Solenopsis wagneri*	Fire Ant	Feeding	None

b. Mode of Action:

Mode of action for TMOF (reported in MRID 455244-07, BPPD Review, September 17, 2003) is as follows: After application to water, TMOF is consumed by mosquito larvae who are

aquatic particulate filter feeders. As the yeast is broken down by digestive processes, TMOF is released into the insect gut. The TMOF protein (a small decapeptide) then passes through the peritropic membrane and midgut wall into the hemolymph. TMOF then binds to specific receptors which, once activated, trigger a halt in trypsin-like enzyme translation (enzyme biosynthesis) and ultimately transcription (mRNA synthesis). The biochemical process of receptor activation is not well understood, though affected insects are unable to produce digestive proteases needed to provide essential amino acids, resulting in starvation.

2. Physical And Chemical Properties Assessment

The physical and chemical characteristics of TMOF were submitted to support the registration. These are summarized in Table 1b.

TABLE 1b. Product chemistry data requirements

GUIDELINE NO.	STUDY	RESULTS	MRID NO.
151-10 (OPPTS 880.1100) 158.155 (OPPTS 830.1550)	Product identity and composition	Acceptable; TMOF, a decapeptide, is expressed in inactivated yeast cells. Mode of action is hormonal disruption of trypsin production leading to starvation.	455244-01 455244-06 455244-07 456049-01 457367-01
151-11 (OPPTS 880.1200) 158.160 (OPPTS 830.1600) 158.162 (OPPTS 830.1620)	Description of starting materials, production and formulation process Description of materials used to produce the product Description of production process	Acceptable; Submitted data satisfies the data requirements for starting materials and manufacturing process. Registrant has submitted an acceptable standardized procedure and confirmatory steps for inactivation (heat-kill) of yeast cells.	455244-03 455244-08 457367-02
151-12 (OPPTS 880.1400) 158.167 (OPPTS 830.1670)	Discussion of formation of impurities	Acceptable; Yeast cells expressing TMOF are inactivated via heat-kill processing and are not known to produce any other secreted proteins. Any contaminated batches will be disposed of by heat sterilization.	455244-02 455244-09
158.170 (OPPTS 830.1700)	Preliminary analysis	Acceptable; Submitted data from 6 batches of TMOF satisfy the requirements for preliminary analysis.	457367-03
158.175 (OPPTS 830.1750)	Certified limits	Acceptable; Certified limits and CSF (dated 10/17/03) satisfy the data requirement.	457367-04

GUIDELINE NO.	STUDY	RESULTS	MRID NO.
158.180 (OPPTS 830.1800)	Enforcement analytical method	Acceptable; TMOF is analyzed using an ELISA test.	457367-05
PHYSICAL / CHEMICAL PROPERTIES FOR the TGAI			
63-2 (OPPTS 830.6302)	Color	Tan	457367-06
63-3 (OPPTS 830.6303)	Physical State	Solid powder	457367-06
63-4 (OPPTS 830.6304)	Odor	Yeast	457367-06
63-5 (OPPTS 830.7200)	Melting point	Not required for MP	N/A
63-6 (OPPTS 830.7220)	Boiling point	Not required for MP	N/A
63-7 (OPPTS 830.7300)	Bulk Density	0.59 g/mL	457367-06
63-8 (OPPTS 830.7840)	Solubility	Not required for MP	N/A
63-9 (OPPTS 830.7950)	Vapor Pressure	Not required for MP	N/A
63-11 (OPPTS 830.7550)	Octanol/water partition coefficient	Not required for MP	N/A
63-12 (OPPTS 830.7000)	pH	5.5	457367-06
63-13 (OPPTS 830.6313)	Stability	Stable at elevated temperature	457367-06
63-15 (OPPTS 830.6315)	Flammability	Not applicable, product is a solid	457367-06
63-17 (OPPTS 830.6317)	Storage stability	In progress - to be submitted as a condition of registration	N/A
63-18 (OPPTS 830.7100)	Viscosity	Not applicable, product is a solid	457367-06

GUIDELINE NO.	STUDY	RESULTS	MRID NO.
63-19 (OPPTS 830.6319)	Miscibility	Not applicable, product is a solid	457367-06
63-20 (OPPTS 830.6320)	Corrosion characteristics	Not corrosive to plastic bags for up to 3 years	457367-06

B. HUMAN HEALTH ASSESSMENT

1. Food Clearances/Tolerances

The proposed pesticide is a manufacturing-use product (Technical TMOF). There is no end use product currently proposed. Therefore, a tolerance or exemption from tolerance was not established concurrently with this action. In addition, end-use products formulated from Technical TMOF will be used for mosquito control in aquatic environments, a non-food use. However, should the registrant seek any food use sites for the end-use product, the requirement of a tolerance must be addressed.

2. Toxicology Assessment

Mammalian toxicology studies have been submitted and are sufficient to support the conditional registration of TMOF for the proposed use as a manufacturing use product. Summaries of the acute toxicological studies (Table 2a) and the rationales for certain data waiver requests (Table 2b) are discussed below.

a. Acute Oral Toxicity (MRID *456049-02*; OPPTS *870.1100)*

Eight male and 8 female mice were dosed with TMOF yeast or untransformed yeast (negative control) via oral gavage at 2000 mg/kg body weight. During the observation period, no TMOF-dosed mice died, although one control mouse died due to injuries during oral gavage. Remaining mice were generally normal in appearance and behavior, although reports of five mice on seven occasions showed altered physical appearance or levels of activity, reactivity, or behavior. Surviving mice all gained weight during the study, although weights declined slightly in the second week of the study for all treatments (there were no observed weight differences between controls and treated mice). Necropsy at study termination showed no macroscopic lesions or evidence of adverse effects. With an LD_{50} > 2,000 mg/kg body weight, the pesticide was classified as Toxicity Category III for acute oral toxicity (MRID# 456049-02; BPPD Review, dated September 17, 2003).

b. Acute Oral Toxicity/Pathogenicity (MRID 457367-09; OPPTS 885.3050)

Although not normally required for biochemical pesticides such as TMOF, an acute oral toxicity/pathogenicity study (OPPTS 885.3050) was submitted due to the fact that TMOF is expressed in a microbe (*Pichia pastoris* yeast) that is heat-inactivated. In the study, 18 albino rats were assigned to one of three treatment groups: 1) negative, non-dosed control group housed in a separate room, 2) negative non-dosed control group housed in the treatment room, and 3) test group exposed to TMOF yeast (presumably 1 ml or 2 x 10^8 cells per animal). During the study, no rats died, all animals gained weight, no test material-related clinical signs or abnormal gross necropsy findings were noted from live TMOF yeast exposure. Infectivity testing was not performed. The oral (gavage) No Observed Effects Dose (NOED) of live TMOF yeast for treated male and female albino rats was likely > 2 x 10^8 cells per mL per animal, although nominal and unmeasured doses were used. The study was rated "Supplemental" due to lack of enumeration of the test substance and no reports of weight or volume for the test dose. However, considering that this is not a required study, the data from this report are regarded as supplemental information to support the registration of TMOF (MRID# 457367-09; BPPD Reviews, dated September 17, 2003 and January 27, 2004).

c. Acute Dermal Toxicity (MRID 456049-03; OPPTS 870.1200)

Five male and 5 female New Zealand white rabbits were exposed to TMOF yeast administered over clipped skin at 5000 mg/kg body weight. There was no mortality, clinical or gross necropsy findings from dermally applied TMOF yeast. Body weights of male and female rabbits increased steadily during this study. All rabbits experienced slight to moderate erythema that subsided by day 8, and very slight edema that subsided by day 2. One rabbit had focal eschar on days 3 and 4. Nine rabbits had desquamation beginning on days 2-4 that cleared by day 9 in eight rabbits, but persisted through day 14 for one rabbit. With a LD_{50} > 5,000 mg/kg body weight, the pesticide is classified as Toxicity Category IV for acute dermal toxicity. Due to the mild irritation with clearance by 8 days, the pesticide is classified as Toxicity Category III for dermal irritation (MRID# 456049-03; BPPD Review, dated September 17, 2003).

d. Acute Inhalation Toxicity (MRID 455244-10; OPPTS 870.1300)

Five male and 5 female Crl:CD (SD)IGS rats exposed to 2.4 mg per L TMOF yeast over four hours, with average total airflow of 77 liters per min in a nose-only exposure chamber volume of 8.6 L. The rats were observed for mortality at the approximate midpoint of exposure on day 0, and twice daily for 14 days. Clinical signs were noted daily for 14 days if they occurred. Rats were weighed prior to exposure, and on days 7 and 14; then euthanized and necropsied on day 14. All rats survived the study and had normal body weight gain. Dried red / tan material around the facial area was noted on all animals following exposure; two females had dried red material around the nose and one female had a black nose on days 1-2; all were normal by day 3. No macroscopic findings were noted on necropsy. With an

LC_{50} of > 2.4 mg/L, the pesticide is classified as Toxicity Category IV for acute inhalation toxicity (MRID# 455244-10; BPPD Review, dated September 17, 2003).

e. Mammalian Cells in Culture Gene Mutation Assay in Mouse Lymphoma L5178Y Cells (MRID 456228-02; OPPTS Guideline 870.5300)

In a mammalian cell gene mutation assay, L5178Y/$tk^{+/-}$ mouse lymphoma cells *in vitro* were exposed to TMOF in distilled water at 4000, 4200, 4400, 4600, 4700, 4800, 4900, or the limit concentration of 5000 µg per mL in presence or absence of mammalian metabolic activation (S9-mix) obtained from Aroclor 1254 induced male Sprague-Dawley rat liver. A preliminary range-finding study at 0.53 to 5360 µg per mL showed no cytotoxicity or precipitation, so a maximum 5000 µg per mL was used for mutagenicity testing. No increase in mutant frequency compared to solvent controls, or dose-response, was observed at any concentration in single cultures. Mutants reached a frequency of 53 x 10^{-6} surviving cells at 4700 µg per mL without S9-mix and 74 x 10^{-6} surviving cells at 4400 µg per mL with S9-mix, with appropriate positive and solvent control responses. Mutant frequency for the MMS positive controls was 1071-1578 x 10^{-6} surviving cells without S9-mix, and for cyclophosphamide with S9-mix 1217-1705 x 10^{-6} surviving cells. TMOF showed no mutagenic activity with or without S9 metabolic activation (MRID# 456228-02; BPPD Review, dated September 17, 2003).

f. Mutagenicity-Reverse Mutation Assay in Bacteria (MRID 456228-01; OPPTS Guideline 870.5100)

Mutagenicity testing is not normally required as part of the Tier 1 toxicity data requirements. However, a mutagenicity-reverse mutation assay (OPPTS 870.5100) was submitted as supplemental information to support the registration. In the submitted reverse gene mutation assay in bacteria, strains TA98, TA100, TA102, TA1535 and TA1537 of *Salmonella typhimurium* were exposed to a range of TMOF to 5000 µg per plate, in the presence and absence of mammalian metabolic activation (S9-mix) with triplicate standard plate tests. Plates were incubated 2-3 days at 37 ºC and revertant colonies were counted by hand. A positive response was a dose-related increase in revertants over three concentrations, or an increase at least twice the solvent control. There was no cytotoxicity with or without metabolic activation at any concentration, and no precipitation was observed. The mutagenicity assay showed no significant increase in the number of revertants per plate for any test strain, with or without S9-mix. There was no evidence of induced mutant colonies over background in *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537. TMOF was tested up to the limit dose (5000 µg per plate) and satisfies OPPTS Guideline 870.5100 for *in vitro* mutagenicity [bacterial reverse gene mutation] data (MRID# 456228-01; BPPD Review, dated September 17, 2003).

g. *In Vitro* Chromosome Aberration Assay in Chinese Hamster Ovary (CHO) Cells (MRID 456228-03; OPPTS Guideline 870.5375)

A chromosome aberration assay is not normally required as part of the Tier 1 toxicity data requirements. However, the registrant submitted an *in vitro* chromosome aberration assay in Chinese hamster ovary (CHO) cells (OPPTS 870.5375) as supplemental information to support the registration. CHO cell cultures were exposed to TMOF in sterile distilled water at 500, 890, 1580, 2810 or the limit dose of 5000 µg per mL, with (19 and 43 hours exposure - 2 hour recovery) and without (4 hours exposure - 17 hour recovery) metabolic activation (S9-mix). Cell cultures not S9-mix treated were harvested at 21 and 45 hours, and cultures treated with S9-mix were harvested after 21 hours. No cytotoxicity, increase in cells with chromosomal aberrations, or increase in chromosomal aberrations per cell compared to controls was observed with or without activation. Cyclophosphamide and mitomycin-C positive control values were acceptable. During the tests TMOF did not significantly alter pH or osmolality of the media so results were considered valid. Therefore, TMOF is evaluated as negative for *in vitro* induction of cytogenetic damage (MRID# 456228-01; BPPD Review, dated September 17, 2003).

h. Hypersensitivity Incidents (No OPPTS Guideline)

The registrant must report to the Agency any incident(s) of hypersensitivity with TMOF under FIFRA Section 6(a)(2).

i. Data Waiver Requests: Health Effects

Data waivers were requested for the following Tier I studies:

(i) Primary Eye Irritation (OPPTS Guideline 870.2400)
(ii) Primary Dermal Irritation (OPPTS 870.2500)
(iii) Dermal Sensitization Study (OPPTS 870.2600)
(iv) Immune Response (OPPTS 880.3550)
(v) 90-Day Studies (OPPTS 870.3100 - 870.3465)
(vi) Teratogenicity Study (OPPTS 870.3700)

The Agency decided that the justifications provided by the applicant to waive the studies listed above, [(i) through (vi)], were acceptable as discussed below [BPPD reviews of Data Waiver Requests, dated September 17, 2003 and January 27, 2004].

Summaries of discussions for Data Waiver Requests

(i) Primary Eye Irritation (OPPTS 870.2400)

The registrant notes that in an initial consultation with EPA February 16, 1995, noted in March 1, 1995 meeting minutes, EPA would require this study on end-use products only.

Since this submission contains a manufacturing use product for registration, the registrant requests a waiver for this study. The registrant also conceded that the label may contain a warning to wear appropriate eye protection when working with TMOF materials, in absence of this test. Therefore, the data waiver request for primary eye irritation testing is granted (BPPD Review - September 17, 2003). The pesticide is considered Toxicity Category III for labeling purposes.

(ii) Primary Dermal Irritation (OPPTS 870.2500)

The registrant notes that a more stringent study, an Acute Dermal Toxicity Study, OPPTS Guideline 870.1200 (MRID 456049-03), was performed at 5000 mg per Kg and for 14 days, on shaved un-abraded skin of rabbits. The 870.1200 test used a longer exposure and higher dosage than required under 870.2500. The results of the acute dermal toxicity study showed that there was no mortality, clinical or gross necropsy findings from dermally applied TMOF yeast. Body weights of male and female rabbits increased steadily during this study. All rabbits experienced slight to moderate erythema that subsided by day 8, and very slight edema that subsided by day 2. One rabbit had focal eschar on days 3 and 4. Nine rabbits had desquamation beginning on days 2-4 that cleared by day 9 in eight rabbits, but persisted through day 14 for one rabbit. The test dose in the acute dermal toxicity study, 5000 mg per Kg, was approximately 2x higher than required for this guideline (500 mg per animal; test animal weight ranges 2,352-2,539g [M] and 2,308-2,521g [F]). As such, the data waiver request for primary dermal irritation testing is granted (BPPD Review - September 17, 2003). The pesticide is considered Toxicity Category III based on the results from the acute dermal toxicity study.

(iii) Dermal Sensitization (OPPTS 870.2600)

The registrant analyzed both the potential allergenicity of *Pichia pastoris* yeast, and of TMOF or related compounds (from a BLAST search of sequences). A literature search (PUBMED, NIH NLM Gateway, AGRICOLA) turned up two references of limited relevance. One refers to use of yeast expression systems to produce recombinant allergens and the other used yeast derivatives as adjuvants for immune response with a cattle vaccine, with no adverse effects reported. An analysis of protein sequences similar to TMOF in a submitted BLAST search was of limited utility given the short TMOF sequence and the hexapeptide poly-proline sequence repeat. This search yielded associations with sequences from microorganisms, viruses, and eukaryote sources ranging from the simplest to the most complex organisms. Literature searches of immunogenicity or antigenicity to these proteins reportedly turned up no references. Analysis of the most similar proteins showed they "were associated with a protein structural function with no reported role in biological activity." The registrant also notes that an OPPTS 870.1200 Acute Dermal Toxicity Study (MRID 456049-03) resulted in a classification of ACCEPTABLE - LD_{50} > 5000 mg per Kg - Toxicity Category IV; Irritant Toxicity Category III (a mild irritant with symptom clearance by 8 days). EPA notes that yeast is on the Minimal Risk Inerts List 4A. As such, the data waiver request for dermal sensitization testing is granted (BPPD Review - January 27, 2004).

Any hypersensitivity or adverse effects incidents that occur with TMOF, in production or use, must be reported to EPA .

(iv) Immune Response (Immunotoxicity Testing) (OPPTS 880.3550)

The registrant notes that in an initial consultation with EPA on February 16, 1995 (noted in the meeting minutes dated March 1, 1995) a waiver based on the completion of a digestibility study that shows degradation of the TMOF decapeptide to mosquito-inactive intermediates would be considered. The digestibility study (MRID 456049-01) shows degradation of TMOF to mosquito-inactive intermediates within 24 hours. Therefore, the data waiver request for the immune response testing is granted (BPPD Review - September 17, 2003).

(v) 90-Day Studies: Feeding (OPPTS 870.3100), Dermal (OPPTS 870.3250), Inhalation (OPPTS 870.3465)

Ninety day studies (feeding, dermal, inhalation) are typically required if the proposed use sites are likely to result in significant human exposure. In the case of TMOF, the product is a manufacturing use product that is unlikely to result in such exposure. In addition, the registrant requested a waiver for these studies based on an initial consultation with EPA on February 16, 1995 (noted in the meeting minutes dated March 1, 1995), in which it was agreed that a waiver based on the completion of a digestibility study showing degradation of the TMOF decapeptide to mosquito-inactive intermediates would be considered. The digestibility study (MRID 456049-01) shows degradation of TMOF to mosquito-inactive intermediates within 24 hours. As such, the requirements for 90-day feeding, dermal, and inhalation testing are waived (BPPD Review - September 17, 2003).

(vi) Teratogenicity Study (OPPTS 870.3700)

The registrant notes that in an initial consultation with EPA on February 16, 1995 (noted in the meeting minutes dated March 1, 1995), in which it was agreed that a waiver based on the completion of a digestibility study showing degradation of the TMOF decapeptide to mosquito-inactive intermediates would be considered. The digestibility study (MRID 456049-01) shows degradation of TMOF to mosquito-inactive intermediates within 24 hours. EPA also notes the following mutagen detecting tests performed by the registrant, all with negative results compared to controls: Mutagenicity-Reverse Mutation Assay in Bacteria (870.5100), Mammalian Cells in Culture Gene Mutation Assay in Mouse Lymphoma L5178Y Cells (870.5300) and *In Vitro* Chromosome Aberration Assay in Chinese Hamster Ovary (CHO) Cells (870.5375). Therefore, the data waiver request for teratogenicity testing is granted (BPPD Review - September 17, 2003).

The mammalian toxicity data for TMOF are summarized in Table 2.

TABLE 2. Mammalian toxicity data requirements

Guideline No.	STUDY	RESULTS	MRID NO.
81-1 (OPPTS 870.1100)	Acute oral toxicity	Acceptable; Mouse LD_{50} > 2000 mg/kg Toxicity Category III	456049-02
152A-10 (OPPTS 885.3050)	Acute oral toxicity/ pathogenicity	Supplemental [a]; Rat NOED > 2 x 10^8 cells/mL	457367-09
81-2 (OPPTS 870.1200)	Acute dermal toxicity	Acceptable; Rabbit LD_{50} > 5,000 mg/kg, mildly irritating Toxicity Category IV (toxicity) Toxicity Category III (irritation)	456049-03
81-3 (OPPTS 870.1300)	Acute inhalation toxicity	Acceptable; Rat LD_{50} > 2.4 mg/L Toxicity Category IV	455244-10
152-17 (OPPTS 870.5300)	Studies to determine genotoxicity (mammalian cells in culture gene mutation assay)	Acceptable; TMOF shows no mutagenic activity	456228-02
152-19 (OPPTS 870.5100)	Mutagenicity testing (mutagenicity-reverse mutation assay) [b]	Acceptable; TMOF shows no mutagenic activity	456228-01
(OPPTS 870.5375)	In Vitro Chromosome Aberration Assay in Chinese Hamster Ovary (CHO) Cells [b]	Acceptable; TMOF is negative for *in vitro* induction of cytogenetic damage	456228-03
81-4 (OPPTS 870.2400)	Primary eye irritation	Waived (see section B.2.i for rationale); Assigned Toxicity Category III for labeling	N/A
81-5 (OPPTS 870.2500)	Primary dermal irritation	Waived (see section B.2.i for rationale); Assigned Toxicity Category III, based on the acute dermal toxicity study (see above)	N/A
81-6 (OPPTS 870.2600)	Dermal sensitization	Waived (see section B.2.i for rationale)	N/A
152-20 (OPPTS 870.3100)	90-day feeding	Waived (see section B.2.i for rationale)	N/A
152-21 (OPPTS 870.3250)	90-day dermal		
152-22 (OPPTS 870.3465)	90-day inhalation		
152-23 (OPPTS 870.3700)	Teratogenicity	Waived (see section B.2.i) for rationale	N/A
152-16 (no OPPTS guideline number)	Hypersensitivity incidents	Incident data must be reported to the Agency	N/A

[a] Study was rated Supplemental, however the acute oral toxicity/pathogenicity study is not required for biochemical pesticides.
[b] These studies are not normally required as part of the Tier 1 toxicity testing for biochemical pesticides, but were provided as supplemental information.

3. Dose Response Assessment

Based on available information and data from the open scientific literature, no toxicity endpoints were identified.

4. Aggregate Exposure and Risk Characterization

a. Dietary

The proposed product, Technical TMOF, is a manufacturing use product with no food uses. Furthermore, end use products will be targeted against mosquito larvae, a use pattern that will result in little if any exposure to food crops. Thus, no residues are expected and exposure via the oral route is unlikely.

In addition, the submitted acute oral toxicity test (MRID 456049-02) showed no toxicity or mortality at the dose tested. No toxicity endpoints were identified. Also, a submitted digestibility study (MRID 456049-01) showed degradation of TMOF to mosquito-inactive intermediates within 24 hours.

b. Other non-occupational

The use of the pesticide product, Technical TMOF, is for manufacturing use only and should result in minimal non-occupational exposure. In addition, TMOF has low mammalian toxicity and no toxicity endpoints were identified.

5. Occupational and Residential Exposure

a. Occupational Exposure and Risk Characterization

The pesticide product, Technical TMOF, is for manufacturing use only. The potential for exposure to the pesticide exists only for manufacturers. Because of low acute mammalian toxicity, worker exposure data on TMOF are not required. The "caution" statement and hazard and first aid statements on the label are sufficient to protect from any adverse reactions that may occur from exposure to TMOF. In addition, the label will bear a dust mask statement (NIOSH N-95, P-95, or R-95 standard) to mitigate any potential risk from the inhalation of microbial proteins.

b. Residential, School and Daycare Exposure and Risk Characterization

No indoor residential, school or daycare uses currently appear on the label. Human exposure to TMOF should not occur in these areas. In the absence of any toxicological endpoints, risk from

the consumption of residues of TMOF from its pesticidal use is not expected for populations in residential, school and day care settings, including infants and children.

6. Drinking Water Exposure and Risk Characterization

The pesticide product, Technical TMOF, is for manufacturing use only and should not result in exposure to drinking water. End use products formulated from Technical TMOF may be applied directly to water for control of mosquito larvae. However, such products will be restricted from finished, treated drinking water sources. In addition, as demonstrated by the submitted data, TMOF has low mammalian toxicity, with no toxicity endpoints (see section B.2).

7. Acute and Chronic Dietary Risks for Sensitive Subpopulations, Particularly Infants and Children

The proposed product, Technical TMOF, is a manufacturing use product with no food uses. Furthermore, end use products will be targeted against mosquito larvae, a use pattern that will result in little if any exposure to food crops. Thus, no residues are expected and exposure via the oral route is unlikely. In addition, the submitted acute oral toxicity test (MRID 456049-02) showed no toxicity or mortality at the dose tested. No toxicity endpoints were identified. Also, a submitted digestibility study (MRID 456049-01) showed degradation of TMOF to mosquito-inactive intermediates within 24 hours.

In this instance, based on all available information, the Agency concludes that TMOF is practically non-toxic to mammals including infants and children. Because there are no threshold effects of concern to infants, children and adults when TMOF is used as labeled, the provision requiring an additional margin of safety does not apply. Further, the provisions of consumption patterns, special susceptibility, and cumulative effects do not apply. As a result, EPA has not used a margin of exposure (safety) approach to assess the safety of TMOF.

8. Aggregate Exposure from Multiple Routes Including Dermal, Oral and Inhalation

There is reasonable certainty that no harm will result from aggregate exposure to residues of TMOF to the U.S. population. The Agency has arrived at this conclusion based on the low level of toxicity and the anticipated lack of exposure to TMOF residues. The oral, dermal, and inhalation toxicity studies showed no acute toxicity, and the risks anticipated from these exposures are considered minimal. The risks from aggregate exposure via oral, dermal and inhalation exposure are a compilation of three low risk exposure scenarios and are negligible. Since there are no threshold effects of concern, the provision requiring an additional margin of safety does not apply. Therefore, EPA has not used a margin of exposure approach to assess the safety of TMOF.

9. Cumulative Effects

The Agency has considered the potential for cumulative effects of TMOF and other substances in relation to a common mechanism of toxicity. These considerations include the possible

cumulative effects of such residues on infants and children. As demonstrated in Section B.2 above, TMOF is non-toxic to mammals. Because no mechanism of toxicity in mammals has been identified for this organism, no cumulative effects from the residues of this product with other related microbial pesticides are anticipated.

10. Risk Characterization

The Agency has considered human exposure to TMOF in light of the relevant safety factors in FQPA and FIFRA. A determination has been made that there is a reasonable certainty that no harm to the U.S. population, including infants and children, will result from aggregate exposure to residues of TMOF due to its use as a pest control agent. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. As discussed in section B.2 above, TMOF is non-toxic to mammals. In addition, the present use sites for TMOF do not include food crops, so the requirement of a tolerance is not applicable.

C. ENVIRONMENTAL ASSESSMENT

1. Ecological Effects Hazard Assessment

Below is a summary of the ecological effects database evaluated in support of this action. The database for studies and information of toxicity of TMOF to non-target organisms are sufficient to allow conditional registration as a manufacturing use pesticide.

a. Toxicity to Non-target Animals

The studies summarized below were submitted by the registrant to support TMOF based upon the nature of the active ingredient and the likely use patterns for a TMOF end product (agreed upon in a September 4, 1998 pre-registration meeting with EPA). Data waiver requests were submitted for the other required studies (see section C.1.b below).

(I) Avian Oral Toxicity Test (MRID 456049-08; OPPTS 850.2100; Gdln 154-6)

The toxicity of TMOF was assessed in 14-day old mallard ducks (Anas platyrhynchos). TMOF yeast in diet was administered via oral gavage at a concentration of 125 mg/kg body weight for five days. No TMOF related toxicological symptoms were noted in any of the test birds during the 30-day test period. No toxic effects were observed in birds based upon analyses of feed consumption, loss of body weight, and gross pathological examination conducted at study termination. The NOEC is >125 mg TMOF yeast/kg body weight. (MRID# 456049-08; BPPD Data Evaluation Report, dated July 2, 2003).

(ii) Daphnid Chronic Toxicity Test (MRID 456049-04 and 457357-08; OPPTS 885.4240; Gdln 154-20)

The toxicity of TMOF yeast to Daphnia magna was assessed in static-renewal and flow-through conditions. For static-renewal, young daphnia were exposed to a TMOF yeast concentration ranging from approximately 3.0 x 10^6 cells/mL to 1.0 x 10^6 cells/mL and monitored for immobilization and other sublethal effects. Monitoring also included day of first brood, number of offspring as well as the length and weight of surviving first generation daphnids at test termination on day 21. Daphnids exposed to the TMOF yeast concentrations during the 21 day exposure period experienced no adverse effects on survival or fitness. The NOEC of TMOF for static-renewal is >1.0 x 10^6 cells/mL. For flow-through conditions, young daphinids were exposed to a single TMOF yeast concentration of 1.026 x 10^6 cells/mL and monitored in the same fashion that was done for the static-renewal test. Survival of first generation daphnids was not significantly different than the control group and growth and offspring produced was greater in the treated group than the control. The NOEC of TMOF for flow-through is 1.026 x 10^6 cells/mL (MRID# 456049-04 and 457357-08; BPPD Data Evaluation Report, dated July 2, 2003).

(iii) Mysid Chronic Toxicity Test (MRID 456049-05; OPPTS 850.13540; Gdln 72-4)

The toxicity of TMOF yeast to mysid shrimp (Americamysis bahia) was assessed in flow-through conditions. Young mysids were exposed to a single TMOF yeast concentration of 1.037 x 10^6 cells/mL and monitored for toxicity and other sublethal effects. Monitoring also included number of offspring as well as the length and weight of surviving first and second generation mysids at test termination on day 28. Survival of mysids exposed to TMOF yeast was 78% at test termination which was not significantly lower than the control (83%). Survival of mysids in the sterile cultural filtrate (48%) and in the killed yeast cells without TMOF (58%) was significantly lower than the control, though it was concluded that something other than TMOF was responsible for the reduced survival. Females in the TMOF yeast and sterile culture filtrate groups produced fewer offspring when compared to females in the control group. The lengths and weights of mysids exposed to the TMOF yeast were comparable to the controls at test end. The NOEC of TMOF yeast to mysids is <1.037 x 10^6 cells/mL and the LOEC is 1.037 x 10^6 cells/mL (MRID# 456049-05; BPPD Data Evaluation Report, dated July 2, 2003).

(iv) Fish Early Life Stage Toxicity Test (MRID 456049-06 and 456049-07; OPPTS 850.1400; Gdln 72-4)

The toxicity of TMOF yeast was assessed in the early life stage of the marine sheepshead minnow (Cyprinodon variegatus) and the freshwater fathead minnow (Pimephales promelas). For the sheepshead minnow, embryos were exposed to a single TMOF yeast concentration of 1.038 x 10^6 cells/mL and then monitored for time to hatch and for health of embryos hatched. Hatched minnows were also exposed to a TMOF concentration of 1.038 x 10^6 cells/mL for 33 days and monitored for mortality as well as health and fitness (e.g. length, dry weight, time to hatch or time to first feeding) of surviving fish. No sublethal effects were observed. Survival of sheepshead embryos in the TMOF treatment was 96% at hatch. Of the hatched fish, 98% had survived at the end of the test. Survival in the control, sterile filtrate, and killed yeast (w/o TMOF) treatment groups was 95 to 99%. Survival of the three control groups at test termination was 95%. Exposure of embryonic, larval and juvenile sheepshead minnows to TMOF yeast for 33 days resulted in survival and growth equal to or greater than the controls. The NOEC of TMOF to sheepshead minnow is >1.038 x 10^6 cells/mL. For the fathead minnow, embryos were exposed to a single TMOF yeast concentration of 1.012 x 10^6 cells/mL and then monitored for time to hatch and for health of embryos hatched. Hatched minnows were also exposed to a TMOF concentration of 1.012 x 10^6 cells/mL for 32 days and monitored for mortality as well as health and fitness. Survival of fathead embryos in the TMOF treatment was 96% at hatch. Of the hatched fish, 73% had survived at the end of the test. Survival in the control, sterile filtrate, and killed yeast (w/o TMOF) treatment groups was 86 to 95%. Survival at test termination was 88% in the control, 65% in the sterile filtrate, and 58% in the killed yeast group. Exposure of embryonic, larval and juvenile fathead minnows to TMOF yeast for 32 days resulted in survival and growth equal to or greater than the controls. The NOEC of TMOF to fathead minnow is >1.012 x 10^6 cells/mL (MRID# 456049-06 and 456049-07; BPPD Data Evaluation Report, dated July 2, 2003).

(v) Aquatic Microcosm Test (MRID 457367-07; No Guideline)

An experiment was conducted to evaluate growth of Pichia pastoris yeast cells containing the TMOF gene in a freshwater pond simulation. Freshwater pond samples (sediment and water column) were inoculated with 1.74 x 10^9 Pichia pastoris cells containing TMOF in a small aquarium. After two weeks, yeast cell counts were only 0-5% of the initial inoculum in the two mid-water columns. Likewise, sediment samples revealed yeast cell counts which were less than 10% of the initial inoculum. Results indicate the simulated freshwater environment used was not a conducive environment for Pichia pastoris cells containing TMOF and did not support reproduction of the yeast cells (MRID# 457367-07; BPPD Data Evaluation Report, dated July 2, 2003).

Table 3a: Eco-Toxicology Summary/Studies Evaluated

Guideline No.	*Study*	*Status, Classification & Comments*	*MRID Nos.*
*154-6 *850.2100*	*Avian Oral Toxicity*	*Acceptable. No TMOF related toxicological symptoms were noted in any of the test birds during the 30-day test period. The NOEC is >125 mg TMOF yeast/kg body weight.*	*456049-08*
*154-20 ** *885.4240*	*Daphnid Chronic Toxicity*	*Acceptable. No adverse effects to TMOF were observed in static-renewal and flow-through testing. The NOEC for static-renewal is >1.0 x 10^6 cells/mL, for flow-through NOEC = 1.026 x 10^6 cells/mL.*	*456049-04 457357-08*
*72-4 ** *850.1350*	*Mysid Chronic Toxicity*	*Acceptable; Survival of mysids exposed to TMOF comparable to control. The NOEC is <1.037 x 10^6 cells/mL; LOEC = 1.037 x 10^6 cells/mL.*	*456049-05*
*72-4 ** *850.1400*	*Fish Early Life Stage Toxicity*	*Acceptable. Exposure of TMOF to sheepshead and fathead minnows resulted in survival and fitness equal to or greater than control groups. Sheepshead minnow NOEC is > 1.038 x 10^6 cells/mL, fathead minnow NOEC is > 1.038 x 10^6 cells/mL.*	*456049-06 456049-07*
No Guideline	*Aquatic Microcosm Test*	*Supplemental***. The tested freshwater environment did not support reproduction of Pichia pastoris yeast cells expressing TMOF.*	*457367-07*

* 885 series = OPPTS Microbial Pesticide Test Guideline Numbers.
** These studies are not normally required for biochemical pesticides but were submitted due to the nature of the active ingredient and the potential end product use sites.
*** The aquatic microcosm test was rated supplemental because it is not a guideline requirement.

b. Data Waivers: Ecological Effects

The following ecological effects studies were waived:
(i) Avian Dietary Toxicity (OPPTS 850.2200; Gdln 154-7)
(ii) Freshwater Fish Acute Toxicity Test (OPPTS 850.1075; Gdln. 154-8)
(iii) Freshwater Invertebrate Acute Toxicity (OPPTS 850.1010; Gdln 154-9)
(iv) Non-target Plant Studies (OPPTS 850.4000; Gdln 154-10)
(v) Non-target Insect Testing (OPPTS 880.4350; Gdln 154-11)

Justifications for data waivers

Rationales for these data waiver requests are summarized below:

(i) Avian Dietary Toxicity (OPPTS 850.2200; Gdln 154-7)

An acute avian oral toxicity test (OPPTS 850.2100) was conducted with the mallard duck, since the intended use of TMOF will be in aquatic environments (MRID 456049-08, see discussion in section III.C.1.a). Ducks were exposed to 1.25 g/kg body weight TMOF yeast and observed for 30 days. TMOF yeast TGAI did not adversely affect the mallard ducks. According to the submitted study, the NOEC for the mallard duck is >125 mg TMOF/kg body weight. Therefore, it is acceptable to waive the avian dietary toxicity test based on the results of the mallard duck 30 day oral dosing study and lack of expected or reported adverse effects (BPPD Review, July 2, 2003)

(ii) Freshwater Fish Acute Toxicity Test (OPPTS 850.1075; Gdln 154-8)

Acceptable early-life stage toxicity tests (OPPTS 850.1400) with the sheepshead minnow (MRID 456049-06) and fathead minnow (MRID 456049-07) were submitted by the registrant to support the registration of TMOF (see discussion in section III.C.1.a). The NOEC of TMOF yeast to both the sheepshead and fathead minnow is >1 x 10^6 cells/mL of TMOF yeast TGAI. Therefore, it is acceptable to waive the freshwater fish acute toxicity testing based on the early-life stage tests and the lack of expected or reported adverse effects (BPPD Review, July 2, 2003).

(iii) Freshwater Invertebrate Acute Toxicity Test (OPPTS 850.1100; Gdln. 154-9)

Acceptable daphnid (OPPTS 885.4240, MRID 456049-04 and 457357-08) and mysid (OPPTS 850.1350, MRID 456049-05) chronic toxicity studies were submitted by the registrant to support TMOF. The NOEC of TMOF yeast to daphnids is 1.0 x 10^6 cells/mL and the NOEC for mysids is <1.037 x 10^6 cells/mL. Based on the results of the submitted chronic toxicity studies and the lack of expected or reported effects, it is acceptable to waive the freshwater invertebrate acute toxicity study (BPPD Review, July 2, 2003).

(iv) Non-target Plant Studies (OPPTS 850.4000; Gdln. 154-10)

An extensive literature and AGRICOLA search did not result in any reports of adverse effects to non-target plants. Only one reference (Tortiglione et al. 2002) covered plants. In this article TMOF was expressed in tobacco plants to control the tobacco budworm, *Heliothis virescens*, and did not cause adverse effects to the tobacco plant. Since TMOF prevents trypsin biosynthesis by invertebrate midgut epithelial cells, no adverse effects to plants are expected. Therefore, it is acceptable to waive the non-target plant studies (BPPD Review, July 2, 2003).

(v) Non-target Insect testing (OPPTS 880.4350; Gdln. 154-11)

A search of AGRICOLA revealed 17 articles on TMOF, five of which reported effects on non-target invertebrates. There were no reports of adverse effects on beneficial invertebrates. Since TMOF will only be applied to aquatic environments, beneficial

terrestrial insects will generally not be exposed. Acceptable studies were conducted with TMOF yeast for the aquatic invertebrate *Daphnia magna* (MRID 456049-04 and 457357-08). *Daphnia magna* is a sensitive surrogate species for aquatic invertebrate testing. The NOEC of TMOF yeast to daphnids is >1.0 x 10^6 cells/mL. In light of the lack of toxicity or reproductive effects, it is acceptable to waive the non-target insect testing (BPPD Review, July 2, 2003).

Table 3b: Eco-Toxicology Summary: Data Waivers

Guideline No.	Study	Status, Classification & Comments	MRID Nos. Reviewed
154-7 *850.2200	Avian dietary toxicity	Waived** No toxicity or adverse effects are expected based on a submitted avian acute oral toxicity study (see discussion in Section III.C.1).	N/A
154-8 *850.1075	Freshwater fish acute toxicity test	Waived** No toxicity or adverse effects are expected based on submitted early-life stage fish acute toxicity tests (see discussion in Section III.C.1).	N/A
154-9 *850.1100	Freshwater invertebrate acute toxicity test	Waived** No toxicity or adverse effects are expected based on submitted chronic daphnid and mysid toxicity tests (see discussion in Section III.C.1).	N/A
154-10 *850.4000	Non-target plant studies	Waived** A literature and AGRICOLA search of TMOF revealed no reports of adverse effects to plants.	N/A
154-11 *880.4350	Non-target insect testing	Waived** A literature and AGRICOLA search of TMOF revealed no reports of adverse effects to beneficial insects. Tests with the indicator species *Daphnia magna* revealed no toxicity or adverse effects.	N/A

* OPPTS Microbial Pesticide Test Guideline Numbers.
** Justifications acceptable, see text above for discussion.

2. Environmental Fate and Ground Water Data

Environmental fate was addressed in the submitted aquatic microcosm study (MRID 457367-07) which showed that viable yeast cells containing TMOF do not reproduce in simulated freshwater environments. It should be noted that TMOF-expressing yeast cells are inactivated (killed) in the final product and will not be viable in the environment. Ground water data are not available

for TMOF. Exposure assessments on this type of product (biochemical pesticide) are not performed unless significant human health or ecological effects issues arise in the Tier I studies for either of these disciplines (40 CFR §158.690 (c) and (d)). Since Tier II studies were not triggered, there is no requirement for environmental fate data.

3. Ecological Exposure and Risk Characterization

The ecological data and waiver discussions (as summarized in section III.C.1 above) support a conclusion of reasonable certainty that no incremental hazards to non-target organisms or to the environment are expected as a result of the intended use of TMOF. The proposed product, Technical TMOF, is a manufacturing use product that is not to be directly applied to the environment. End products formulated from TMOF will be applied directly to water for control of mosquito larvae. Five aquatic non-target toxicity tests were submitted (covering four indicator species), all showing no toxicity or adverse effects from TMOF. A separate aquatic microcosm study showed that a *freshwater environment was not a conducive environment for Pichia pastoris yeast cells containing TMOF and did not support reproduction of the yeast cells.*
No further testing for ecological effects or environmental expression is necessary for TMOF.

D. EFFICACY DATA

No efficacy data were required to be submitted to the Agency, since the proposed product, Technical TMOF, is a manufacturing use product that will not be directly applied for control of pests. However, end use products formulated from TMOF are to be targeted against mosquito larvae, a public health pest. Any such end use product will require efficacy data to support mosquito control uses prior to registration.

IV. PUBLIC INTEREST FINDING

The Agency believes the use of TMOF under this conditional registration would be in the public interest. The criteria for Agency evaluation of public interest findings are outlined in 51 FR No. 43, Wednesday March 5, 1986. Under part IV.A, the proposed product may qualify for an automatic presumptive finding that the proposed conditional registration is in the public interest if it is for a minor use, is a unique replacement for pesticides of concern, or is for use against a public health pest.

End products formulated from TMOF will be targeted against mosquito larvae, a public health pest. Therefore, TMOF qualifies for the automatic presumptive finding and is in the public interest.

V. RISK MANAGEMENT DECISION

A. DETERMINATION OF ELIGIBILITY

Section 3(c)(7)(C) of FIFRA provides for the conditional registration of a pesticide containing a new active ingredient (i.e, not contained in any currently registered pesticide) "for a period reasonably sufficient for the generation and submission of required data . . . on the condition that by the end of such period the Administrator receives such data and the data do not meet or exceed risk criteria" identified in regulations issued under FIFRA "and on such other conditions as the Administrator may prescribe." Such a conditional registration will be granted "only if the Administrator determines that use of the pesticide during such period will not cause any unreasonable adverse effect on the environment, and that use of the pesticide is in the public interest."

TMOF is eligible for a conditional registration because its proposed use as a manufacturing use product is in the public interest, and TMOF is not likely to pose any unreasonable risk to health or the environment as discussed in this document. Certain conditions apply to this eligibility and the applicant must take certain actions (e.g., generate and provide certain data) within the time frames outlined in Section VI of this document.

B. REGULATORY POSITION

1. Conditional Registration

Based on the data submitted, BPPD recommends that TMOF, as it is formulated into the manufacturing use product Technical TMOF, is eligible for conditional registration under Section 3(c)(7)(C) of FIFRA. BPPD foresees no adverse effects to human health or the environment from the use of TMOF as an active ingredient. While the biological activity and chemistry of the active ingredient have been adequately described, the registrant has indicated that the storage stability study (OPPTS 830.6317, part of the Physical and Chemical Properties) is ongoing. Therefore, as a condition of registration, the Agency requires submission of the completed storage and stability study.

2. Tolerance for Food Uses and/or exemption

A tolerance is not required for Technical TMOF because the product is a manufacturing use product with no food use sites. End use products formulated from TMOF will be targeted against mosquito larvae in aquatic environments. Should any of the use sites for TMOF end use products involve food uses, a tolerance or an exemption from tolerance will be required.

3. Codex Harmonization

There are no Codex harmonization considerations since there are currently no Codex tolerances for TMOF residues.

4. Nonfood Re/Registrations

There are no nonfood issues at this time. This is a new active ingredient and, therefore, not the subject of reregistration at this time.

5. Risk Mitigation

There is minimal or negligible potential risk to non-target organisms (plants and wildlife), and to ground and surface water contamination through the proposed use of products containing TMOF as discussed in this document. No mitigation measures are required at this time for dietary risk, including risk due to exposure via drinking water. The label will contain a dust mask (NIOSH N-95, P-95, or R-95 standard) statement to mitigate any potential risk from inhaling microbial proteins. The product label will bear Environmental Hazards text to mitigate any potential risk as determined by reviewed data and use sites.

6. Endangered Species Statement

Currently, the Agency is developing a program (The Endangered Species Protection Program) to identify all pesticides whose use may cause potential adverse impacts on endangered and threatened species and their habitats. To aid in the identification of threatened and endangered species and their habitats, several companies have formed an Endangered Species Task Force (EST) under the direction of Crop Life America. Moreover, the EST will assist in providing species location information at the subcounty level, and particularly if an endangered species occurs in areas where pesticides would be used. This information will be useful once the Endangered Species Protection Program has been implemented.

The Agency has no evidence to believe that any endangered or threatened species will be adversely affected by Technical TMOF, a manufacturing use product. End products containing TMOF should not be applied in areas where endangered aquatic invertebrates occur until a thorough review of endangered aquatic invertebrate species is performed.

C. LABELING RATIONALE

It is the Agency's position that the labeling for Technical TMOF, containing 1.1% trypsin modulating oostatic factor, complies with the current pesticide labeling requirements.

1. Human Health Hazard

a. Worker Protection Standard

This product does not come under the provisions of the Worker Protection Standards (WPS) because it is a manufacturing use product.

b. Non-Worker Protection Standard

There are no non-WPS human health hazard issues.

c. Precautionary Labeling

The Agency has examined the toxicological data base for TMOF and concluded that the proposed precautionary labeling of the manufacturing use product, Technical TMOF (i.e. Signal Word, First Aid Statements and other label statements) adequately mitigates the risks associated with the proposed uses.

Manufacturing Use Product Precautionary Labeling: For Technical TMOF: "Caution". "KEEP OUT OF REACH OF CHILDREN." "Harmful if swallowed. Causes moderate eye irritation. Avoid contact with skin, eyes, or clothing. Wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet."

Also, the following dust mask statement will be included on the label: "Mixer/loaders and applicators must wear a dust/mist filtering respirator meeting NIOSH standards of at least N-95, R-95, or P-95. Repeated exposure to high concentrations of microbial proteins can cause allergic sensitization."

d. Spray Drift Advisory

No spray drift advisory statement is necessary for this use.

2. Environmental Hazards Labeling

Manufacturing Use Product Environmental Hazards Labeling: For Technical TMOF: "Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans, or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the local sewage treatment plant authority. For guidance contact your State Water Board or Regional Office of the EPA."

3. Application Rate

There is no application rate specified for the manufacturing use product.

D. LABELING

Product name: Technical Trypsin Modulating Oostatic Factor (TMOF)

Active Ingredient:
Trypsin Modulating Oostatic

Factor (TMOF)	1.1%
Other Ingredients:	98.9%
Total	100.0%

The product labels shall contain the following information:

- Product Name
- Ingredient Statement
- Registration Number
- "Keep Out of Reach of Children"
- Signal Word (CAUTION)

VI. ACTIONS REQUIRED BY REGISTRANTS

Reports of incidents of adverse effects to humans or domestic animals are required under FIFRA, Section 6(a)(2) and incidents of hypersensitivity under 40 CFR Part 158.690(c), guideline reference number 152-16. There are no data requirements, label changes and other responses necessary for the reregistration of the end-use product since the product is being registered after November 1984 and is, therefore, not subject to reregistration. For the same reason, there are also no existing stocks provisions at this time. Before releasing these products for shipment, the registrant is required to provide appropriate labels and other Agency requirements as discussed in this BRAD. The applicant must provide the following data within 30 months of the conditional registration date as shown below in Table 4.

1. Guideline 151-17 Physical and Chemical Properties: Storage Stability (OPPTS 830.6317)

A completed storage stability study is required. The registrant has indicated that the storage stability study was ongoing at the time of the registration application (MRID 457367-06). Therefore, the storage stability study will be a condition of registration and must be submitted within the time frames noted in Table 4 of this BRAD (within 24 months of the date of this conditional registration action).

Table 4: Data required

Guideline	*Title of Study*	*Data required*	*Date due*
**830.6317*	*Storage Stability*	*Completion of the ongoing storage stability.*	*Within 24 months after conditional registration date.*

**OPPTS Harmonized Guidelines*

VII. APPENDIX A

Table 5 lists the use sites for the product.

Table 5: Use Sites

TMOF Use Sites Manufacturing use only. The manufacturing use product is intended for incorporation into end-use products for control of mosquito larvae.	Official date registered:

VIII. REFERENCES

CITATIONS/BIBLIOGRAPHY

Studies submitted in support of this registration action

45524400 Insect Biotechnology, Inc. (2001) Submission of Product Chemistry Data in Support of the Application for Registration of Technical Trypsin Modulating Oostatic Factor. Transmittal of 10 Studies. 28-Sep-2001

45524401 Brandt, A. (2001) Amino Acid Sequence Comparison of Skeetercide (TM Pending) TGAI To Existing Protein Databases. Unpublished study prepared by Insect Biotecnology, Inc. 147 p. 28-Sep-2001

45524402 Groleau, D. (2001) Identification of the Microbial Contaminants of Fermentation 150-02: Supplemental Report. Unpublished study prepared by Microcheck, Inc. 16 p. 28-Sep-2001

45524403 Brandt, A. (2001) Agar Plate Detection Limit Test for TMOF--Pichia pastoris (Skeetercide): Supplemental Report. Unpublished study prepared by Insect Biotechnology, Inc. 11 p. 28-Sep-2001

45524404 Levesque, M. (2001) Heat-Kill Results for Pichia Pastoris-TMOF Yeast: Supplemental Report. Unpublished study prepared by Insect Biotechnology, Inc. 5 p. 28-Sep-2001

45524405 Levesque, M. (2001) Five Day Fermentation of Killed Pichia Pastoris-TMOF Yeast Cells: Supplemental Report. Unpublished study prepared by Insect Biotechnology, Inc. 4 p. 28-Sep-2001

45524406 Brandt, A. (2001) Pichia pastorsis Historical Information: Supplement Report. Unpublished study prepared by Insect Biotechnology, Inc. 128 p. 28-Sep-2001

45524407 Brandt, A. (2001) Product Identity and Composition of Skeetercide (TM Pending) TGAI. Unpublished study prepared by Insect Biotechnology, Inc. 10 p. {OPPTS 880.1100} 28-Sep-2001

45524408 Brandt, A. (2001) Description of Starting Materials, Production and Formulation Process for Skeetercide (TM Pending) TGAI. Unpublished study prepared by Insect Biotechnology, Inc. 11 p. {OPPTS 880.1200} 28-Sep-2001

45524409 Brandt, A. (2001) Discussion of Formation of Impurities of Skeetercide (TM Pending) TGAI. Unpublished study prepared by Insect Biotechnology, Inc. 8 p. {OPPTS 880.1400} 28-Sep-2001

45524410 Kiplinger, G. (2001) Acute Nose-Only Inhalation Toxicity Study of TMOF_Yeast in Albino Rats: Final Report: Lab Project Number: WIL-414001. Unpublished study prepared by WIL Research Laboratories, Inc. 67 p. {OPPTS 870.1300} 28-Sep-2001

45604900 Insect Biotechnology, Inc. (2001) Submission of Toxicity and Environmental Fate Data in Support of the Application for Registration of Technical Trypsin Modulating Oostatic Factor. Transmittal of 8 Studies. 28-Sep-2001

45604901 Thompson, D. (2001) Degradation of TMOF by Digestive Proteases: Final Report. Unpublished study prepared by North Carolina State University. 45 p. 28-Sep-2001

45604902 Thompson, D. (2001) Acute Oral Toxicology of Trypsin Modulating Oostatic Factor (TMOF): Final Report: Lab Project Number: 99-042-B. Unpublished study prepared by North Carolina State University. 22 p. {OPPTS 870.1100} 28-Sep-2001

45604903 Morris, T. (2001) Acute Dermal Toxicity Study of TMOF_Yeast in Albino Rabbits: Final Report: Lab Project Number: WIL-414002. Unpublished study prepared by WIL Research Laboratories, Inc. 41 p. {OPPTS 870.1200} 28-Sep-2001

45604904 Thompson, D. (2001) Daphnid Chronic Toxicology of Trypsin Modulating Oostatic Factor (TMOF): Final Report. Unpublished study prepared by North Carolina State University. 21 p. {OPPTS 850.1300} 28-Sep-2001

45604905 Ward, T.; Boeri, R. (2001) TMOF Yeast: Flow-Through Chronic Toxicity to the Mysid, Americamysis bahia: Lab Project Number: 2155-IB. Unpublished study prepared by T.R. Wilbury Laboratories, Inc. 59 p. {OPPTS 850.1350} 28-Sep-2001

45604906 Ward, T.; Boeri, R. (2001) TMOF Yeast: Early Life Stage Toxicity to the Sheepshead Minnow, Cyprinodon variegatus: Lab Project Number: 2153-IB. Unpublished study prepared by T. R. Wilbury Laboratories, Inc. 40 p. {OPPTS 850.1400} 28-Sep-2001

45604907 Ward, T.; Boeri, R. (2001) TMOF Yeast: Early Life Stage Toxicity to the Fathead Minnow, Pimephales promelas: Lab Project Number: 2154-IB. Unpublished study prepared by T.R. Wilbury Laboratories, Inc. 41 p. {OPPTS 850.1400U 28-Sep-2001

45604908 Thompson, D. (2001) Avian Oral Toxicology of Trypsin Modulating Oostatic Factor (TMOF): Final Report. Unpublished study prepared by North Carolina State University. 31 p. {OPPTS 885.4050} 28-Sep-2001

45622800 Insect Biotechnology, Inc. (2001) Submission of Toxicity Data in Support of the Application for Registration of Technical Trypsin Modulating Oostatic Factor (TMOF). Transmittal of 3 Studies. 28-Sep-2001

45622801 Eash, M. (1999) Microbial Mutagenesis Testing of TMOF Using the Salmonella typhimurium Plate Incorporation Test With and Without Metabolic Activation (The Ames Test): Final Report: Lab Project Number: 99146. Unpublished study prepared by Genesys Research, Inc. 33 p. {OPPTS 870.5100} 28-Sep-2001

45622802 Eash, M. (1999) In Vitro Forward Mutation Assay of TMOF Using the L5178Y/tk+/- Mouse Lymphoma Cell Mutagenesis Assay (MLA) with Colony Sizing, With and Without Metabolic Activation: Final Report: Lab Project Number: 99147. Unpublished study prepared by Genesys Research, Inc. 37 p. {OPPTS 870.5550} 28-Sep-2001

45622803 Eash, M. (1999) In Vitro Chromosomal Aberration Assay of TMOF in Chinese Hamster Ovary (CHO) Cells, With and Without Metabolic Activation: Final Report: Lab Project Number: 99148. Unpublished study prepared by Genesys Research, Inc. 39 p. {OPPTS 870.5395} 28-Sep-2001

45736700 Insect Biotechnology, Inc. (2002) Submission of Product Chemistry, Product Performance, Risk, Exposure, Environmental Fate and Toxicity Data in Support of the Registration of Technical Trypsin Modulating Oostatic Factor. Transmittal of 9 Studies. 15-Aug-2002

45736701 Beegle, C. (2002) Description of Host Range of Skeetercide TGAI. Unpublished study prepared by Insect Biotechnology, Inc. 7 p. {OPPTS 880.1100} 15-Aug-2002

45736702 Beegle, C. (2002) Description of Starting Materials, Production and Formulation Process for Skeetercide TGAI: Product Identity: Supplemental Report. Unpublished study prepared by Insect Biotechnology, Inc. 33 p. {OPPTS 880.1200} 15-Aug-2002

45736703 Beegle, C. (2002) Preliminary Analysis of Skeetercide TGAI. Unpublished study prepared by Insect Biotechnology, Inc. 21 p. {OPPTS 830.1700} 15-Aug-2002

45736704 Beegle, C. (2002) Certified Limits of Skeetercide TGAI. Unpublished study prepared by Insect Biotechnology, Inc. 5 p. {OPPTS 830.1750} 15-Aug-2002

45736705 Beegle, C. (2002) Enforcement Analytical Method for Skeetercide TGAI. Unpublished study prepared by Insect Biotechnology, Inc. 18 p. {OPPTS 830.1800} 15-Aug-2002

45736706 Beegle, C. (2002) Description of Physical and Chemical Properties of Skeetercide TGAI: Supplemental Report. Unpublished study prepared by Insect Biotechnology, Inc. 14 p. {OPPTS 830.6302, 830.7300, 830.6304, 830.7000, 830.6313, 830.6317, 830.6320} 15-Aug-2002

45736707 Groleau, D. (2002) Survival of Pichia pastoris Cells (Pichia-TMOF, Clone 2) in an Environment Simulating a Freshwater Pond Environment: Supplemental Report. Unpublished study prepared by National Research Council Canada Biotechnology Research Institute. 10 p. 15-Aug-2002

45736708 Boeri, R.; Wyskiel, D.; Ward, T. (2002) Live Frozen TMOF Yeast: Flow-Through Chronic Toxicity to the Daphnid, Daphnia magna: Lab Project Number: 2346-IB. Unpublished study prepared by T.R. Wilbury Laboratories, Inc. 44 p. {OPPTS 850.1300} 15-Aug-2002

45736709 Hurley, J. (2002) Acute Oral Toxicity Study of Live TMOF Yeast in Albino Rats: Final Report: Lab Project Number: WIL-414003. Unpublished study prepared by Wil Research Laboratories, Inc. 77 p. {OPPTS 885.3050}

Federal Register Publications

1. *Federal Register: October 9, 2002 (Volume 67, Number 196) (Notices) (Page 62965-62967). Trypsin Modulating Oostatic Factor (TMOF); Pesticide Products; Registration Application.*

2. *Registration of a New Active Ingredient (to be published in 2004).*

BPPD Data Evaluation Records/Reviews

Robyn Rose and Zigfridas Vaituzis, U.S. EPA, OPP/BPPD. July 2, 2003. Review of Ecological toxicity studies including Daphnia Chronic Toxicity, Mysid Chronic Toxicity, Fish Early-Life Stage Toxicity, Avian Dietary Toxicity and Aquatic Microcosm for Trypsin Modulating Oostatic Factor. Review of waiver requests for Avian Dietary Toxicity, Freshwater Fish LC50, Non-target Plant and Non-target Insect testing (MRID# 455244-06, 456049-04, 456049-05, 456049-06, 456049-07, 456049-08, 457367-07, and 457367-08).

Joel Gagliardi and John Kough, U.S. EPA, OPP/BPPD. September 17, 2003. Review of submitted studies, background material and waiver requests to support registration of the manufacturing use product Skeetercide containing heat-killed Pichia pastoris yeast with integral Trypsin Modulating Oostatic Factor (TMOF). (MRID# 455244-01, 455244-02, 455244-03, 455244-06, 455244-07, 455244-08, 455244-09, 455244-10, 456049-01, 456049-02, 456049-03, 456228-01, 456228-02, 456228-03, 457367-01, 457367-02, 457367-03, 457367-04, 457367-05, 457367-06, 457367-09).

Joel Gagliardi and John Kough, U.S. EPA, OPP/BPPD. January 27, 2004. Review of submitted studies, background material and waiver requests to support registration of the manufacturing use product Skeetercide containing heat-killed Pichia pastoris yeast with integral Trypsin Modulating Oostatic Factor (TMOF). (MRID# 455244-02, 455244-07, 455244-08, 455244-09, 456049-01, 457367-01, 457367-02, 457367-03, 457367-04, 457367-05, 457367-06, 457367-09).