Primary Reviewer: Nasrin Begum, Ph.D. Signed: December 10, 2006
Tetrahedron Contractor

EPA Secondary Reviewer: __Angela L. Gonzales Date __01/12/07

STUDY TYPE: Efficacy Study: OPPTS Guideline 810.3400

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SUBMISSION: Reg. No. 75318-10

TEST MATERIAL: B2E-10

MRID No.: 469511-01

CITATION: Sjorgren, R.D. (2006). Field Evaluation of B2E-10 Against *Ochlerotatus melanimon* Mosquitoes in the Owen Valley of California

SUBMITTER: B2E Biotech LLC
3330 Noyac Road
Sag Harbor, NY 11963

EXECUTIVE SUMMARY: Plots of native irrigated pasture of mosquito (*Ochlerotatus melanimon)* breeding site were treated by tossing B2E-10 Water Soluble Pouch (WSP) in the lowest area of dry sites 21 to 27 days before irrigation. Upon contact with water, the WSP dissolved and released buoyant content to dispose across the water surface throughout the site. The average discharge rate was 1.8 WSP/5753 ft^2 of test area. Ninety nine percent control was achieved, this was determined by pupal collection of the sites and assessed by adult emergence completion of pupal development.

COMPLIANCE: Signed and dated Good Laboratory Practice (GLP) and No Data Confidentiality Claims statements were provided. The GLP statement stated the study had been conducted in accordance with 40 CFR Part 160 with the following exception: the stability, characterization, identity and verification of the test substance concentration were not reported. A Quality Assurance statement was not included in the report.

CLASSIFICATION: ACCEPTABLE

I. MATERIALS AND METHODS

A. Test Organism: *Ochlerotatus melanimon* mosquito larvae (occur naturally in the native irrigated pasture sites)

B Test Material: B2E-10 (1.8% Methoprene)

C. Test sites

Test plots ware identified by the Owens Valley Mosquito Control Program staff as known mosquito (*Ochlerotatus melanimon)* breeding sites that flood periodically. Dimensions of each of the test sites were measured to calculate the correct dosage rate for the test sites. Five sites are selected and the specifications are listed in Table1.

1. Site 1: Five Willow Cuts, Diversion 9, N. of Wye Road, Bishop, California.
2. Site 2: Single Cottonwood (West of Two Cuts), Diversion 9, N. of Wye Road, Bishop, California.
3. Site 3: Irrigation canal of Reynolds Road, 1.5 mile North of Big Pine, California.
4. Site 4: Irrigation canal of Reynolds Road, 1 mile North of Big Pine, California.
5. Site 5: Gate 5A West, native irrigation site 100 yds south of Site 3, Bishop, California

D. Treatment:

Site1. This 1.83 acre native irrigated pasture site was treated pre-hatch on August 16, 2006 at the rate of 1 WSPs (1/5,472 sq.ft.) on damp mud in the lowest areas of the site.

Site 2. This 0.13 acre native irrigated pasture site was treated pre-hatch on August 16, 2006 at the rate of 14 WSPs (1/5,694 sq.ft.) in the lowest areas of the site.

Site 3. This 0.25 acre native irrigated pasture site was treated pre-hatch on August 16, 2006 at the rate of 2 WSPs (1/5,500 sq.ft.) on damp mud in the lowest areas of the site.

Site 4. This 0.14 acre native irrigated pasture site was treated pre-hatch on August 16, 2006 at the rate of 1 WSP (1/6,000 sq.ft.) to dry ground in the lowest areas of the site.

Site 5. This 0.30 acre native irrigated pasture site was treated on August 25, 2006 at the rate of 10 WSPs (1/6,444 sq.ft.) and were placed to age in dry area in full sunshine.

E. Control Efficacy: Efficacy of B2E-10 was assessed by monitoring the irrigation cycle to determine the elapsed time between treatment and flooding, and thereafter the development of larvae in each respective field site until pupation. Thirty pupae were collected wherever possible from each site perimeter and placed in new Smart and Final

(Los Angeles 90051) Iris Plastifoam 12 oz. Food Containers. Pupae development outcome was recorded (i.e. pupae dead in cup, adult mosquito dead on water, adult mosquito flew from cup) once containers were properly labeled and properly shipped to the laboratory

II. DATA COLLECTION AND EVALUATION

Site1: On September 6, 2006, 21 days later, irrigation flooded the site again. During the brood development, based on the dip counts, it is estimated that >30% of larvae were dead. When surviving larvae had developed to pupae, two collections of 62 and 73 pupae respectively collected on September 27, and monitored for adult emergence, only 3 pupae survived but died as adult, showing 100% inhibition of emergence.

Site 2: On September 6, 2006, 21 days later, irrigation flooded the site again. During the brood development, based on the dip counts, it is estimated that most of the larvae were dead. When surviving larvae had developed to pupae, 36 pupae were collected on September 17 and monitored for adult emergence. Based on low immature survival (21 dead pupae, 11 dead adults, 4 live adults on water), the combined inhibition of emergence was estimated at >95%.

Site 3: On September 12, 2006, 27 days later, irrigation flooded the site again. During the brood development, present at an average of 20/dip in 3rd instar, it is estimated that half of the larvae were dead/moribund. Inspection on September 17, site dried down to 5% original moisture content, 100% larval mortality resulted from the treatment.

Site 4: On September 12, 2006, 27 days later, irrigation flooded the site again. During the brood development present at an average of 20/dip in 3rd instar stage on September 15, it is estimated that half of the larvae were dead/moribund. Inspection on September 17, site dried down to <5% original moisture content, 100% larval mortality resulted from the treatment.

Site 5: On September 16, 2006, 22 days later in the sun, placed in plastic bag and post-hatch treatment, 3rd and 4th instar observed on September 19. On September 15, it is estimated that half of the larvae were dead/moribund. Inspection on September 17, only 8 pupae were developed, and upon completion of pupal development 100% inhibition of emergence was estimated from the treatment.

III. RESULTS

Results are reported as inhibition of emergence (±/SE) on elapsed days post treatment required for the group to complete development. Data were analyzed using a chi-square test at 0.05 levels between control and treatment. The results of the study are summarized in Table 1 and Table 2. The data in the tables are not transformed.

Table 1. Summary of the B2E-10 WSP Treatments on *Ochlerotatus melanimon* in Native Irrigated Pasture Depressions, Owen Valley, CA

Site#	Site Description	Species present	Site Size (acre)	Site Depth (inch)	Application Rate	Days Prehatch	% Inhibition Emergence (IE)
1	Irrigated Pasture	*O. melanimon*	1.83	24	14 WSP	21	100%
2	Irrigated Pasture	*O. melanimon*	0.13	16	1 WSP	21	>95%
3	Irrigated Pasture	*O. melanimon*	0.25	24	2 WSP	27	100%
4	Irrigated Pasture	*O. melanimon*	0.14	20	1 WSP	24	100%
5	Irrigated Pasture	*O. melanimon*	0.30	18	2 WSP	22	100%

Table 2. Inhibition of Emergence of *Ochlerotatus melanimon* by B2E-10 WPS Treatment in Field Sites (August 16, 2006- September 28, 2006, Owens Valley, CA

	Control				B2E-10		
Site#	Pupae	Adult	IE%	Sites	Pupae	Adults	% Inhibition Emergence (IE)
1	44	44	0	1	135	0	100%
2	27	27	0	2	36	4	>95%
3	29	29	0	3	0	0	100%**
4	39	37	5	4	0	0	100%**
5	20	19	5	5	8	0	100%
6	25	22	12				
Total	184	178	3.3±1.32		179	4	97.8±1.10

The overall difference in IE% by chi-square test was highly significant (Chi square test = 324.14, p,,,0.001). ** by larval mortality

IV. DISCUSSION

No major guideline deviation exists.