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EEB REVIEW

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TYPE PRODUCT Miticide

PRODUCT MANAGER G. LaRocca(15)

PRODUCT NAME Agrimec 0.15 EC (Avermectin)

COMPANY NAME Merck Sharp & Dohme

SUBMISSION PURPOSE Submission of Laboratory Toxicity Data and Registrants Wild Mammalian and Aquatic Risk Assessment for Cotton and Citrus

SHAUGHNESSEY NO.	CHEMICAL	%AI
122804	Abamectin	

ECOLOGICAL EFFECTS BRANCH REVIEW

Avermectin

100 Submission Purpose and Label Information

100.1 Submission Purpose and Pesticide Use

The registrant, Merck Sharp and Dohme, proposes to register Abamectin for use on Cotton and Citrus. They are also responding to EEB's concern for hazards to mammals (408563-03) and aquatic organisms (408563-02).

100.2 Formulation Information

Agrimec 0.15 EC is 2 % Avermectin B1, 1 gallon contains 0.15 lb. ai.

100.3 Application Methods, Direction, Rates

(From previous reviews)

Citrus

Aerial application is permitted. Use rate is 0.0125 to 0.025 lb. ai/acre. The label indicates up to 3 applications per season are permitted. No between treatment interval is specified, however, the registrant indicates that the interval would typically be 60 days.

Cotton

Label only indicates ground application is permitted. Use rate is 0.01 to 0.02 lb. ai/acre. The label indicates up to 3 applications per season are permitted. No between treatment interval is specified, however, the registrant indicates that the interval would typically be 21 days.

In a discussion on page 22 of Merck's discussion of mammalian hazard (408563-03), they indicate that residual control of the product is about 90 days from a summer or fall spray. This same statement is repeated on page 18 of their discussion of aquatic risk (408563-02).

100.4 Target Organism

Mites

100.5 Precautionary Labeling

No labeling was provided with this action, however, the following statement was on labels of previous submissions:

"This product is toxic to fish and wildlife. Do not apply directly to water. Do not contaminate water by cleaning of equipment or disposal of wastes. Do not apply when weather conditions favor drift from target area.

This product is highly toxic to bees exposed to direct treatment or residues on blooming crops or weeds. Do not apply this product or allow drift to blooming crops or weeds if bees are visiting the treatment area."

101 Hazard Assessment

101.1 Discussion

Maximum application rate is at 0.025 lb. ai/acre for citrus and 0.02 for cotton. The maximum number of applications would be 3 per season at an unspecified interval. Citrus and cotton are grown in areas associated to estuarine and marine habitat. Citrus groves tend to be flat and sandy. Both citrus and cotton provide habitat for small mammals.

101.2 Likelihood of Adverse Effects on Nontarget Organisms

The following summarizes the known toxicity information on avermectin.

Acute Tests

Bobwhite quail	LD50>2000 mg/kg
Mallard duck	LD50= 85 mg/kg
Bobwhite quail	LC50=3102 ppm
Mallard duck	LC50= 383 ppm
Mouse	LD50= 13-23 mg/kg
Rat	LD50= 10-11 mg/kg
Nonpolar metabolite / rat	LD50> 48 mg/kg
Polar metabolite / rat	LD50>5000 mg/kg
Weanling rat	LD50=1.5 mg/kg
10-day oral pregnant mouse test 77-717-1	NOEL=0.05 mg/kg/day (0.25 ppm1) LEL=0.075 mg/kg/day (0.375 ppm[1]) (1 of 20 female mice died at both 0.1 [3 days] and 0.075 [4 days] mg/kg)
Bluegill	LC50=9.6 ppb
Rainbow trout	LC50=3.2 ppb

Daphnia magna	LC50 0.22-0.34 ppb
Avermectin B1a	0.42 ppb)
Polar metabolite	4.2 ppb)
Moderately polar metabolite	6.3 ppb) > degradates of abamectin
Nonpolar metabolite	25.4 ppb)
Thin-film polar metabolite	76.7 ppb)
8 a-hydroxy avermectin B1a	25.5 ppb)
Mysid shrimp	LC50= 0.02 - 0.033 ppb
Sheepshead minnow	LC50= 15 ppb
Oyster embryo-larvae	LC50= 430 ppb
Earthworm (Eisenia foetida)	LC50= 18 ppm 28-day 33 ppm 14-day 62 ppm 7-day
Chronic Tests	
Rat 1-generation reproduction 77-712-0	NOEL=0.1 mg/kg/day (0.5 ppm[1]) LEL=0.2 mg/kg/day (1 ppm[1])
Rat 1-generation reproduction 77-706-0	NOEL<0.5 mg/kg/day (2.5 ppm[1]) (decreased pup survival, delay in eye-opening)
Mouse Teratogenic effects test 76-723-3	LEL=0.2-0.4 mg/kg/day (1-2 ppm[1])
Mouse Terat. with photodegradate 84-722-1	NOEL=0.05 mg/kg/day (0.25 ppm[1]) LEL=0.1 mg/kg/day (0.5 ppm[1])
Avian reproduction test	NOEL=12 ppm LEL=64 ppm (reduced egg prod.)
Daphnia magna life-cycle	MATC >0.03<0.09 ppb (all dead by day 5 at 0.09 ppb)
Rainbow trout early life stage	MATC >0.52<0.96 ppb
Mysid Shrimp Life-cycle	MATC >0.0035<0.0093 ppb

[1] Assuming a small mammal consumes 20% of its body weight per day. Many mammals such as voles, mice, rats and shrews commonly ingest at least 20% of their weight per day. Exposure may also occur through other routes such as grooming and licking fur or feet. PPM=dose mg/kg / % of body wt. consumed per day.

Summary of Environmental Fate Information

(From Draft Pesticide Fact Sheet No. 84 for Avermectin B1)

Solubility:	7.8 ppb	
Octanol/Water Partition Coefficient:	9,900	
Photolyses:	$t^1/_2$ < 1 day	
Hydrolysis:	minimal	
Soil metabolism:	Aerobic $t^1/_2$ approx. 2 months Anaerobic is slower	
Leaching tendency:	minimal	
Accumulation:	Bluegill	69X whole fish 30X fillet 110X viscera

An aquatic degradation and fate study[2] indicated that once it reaches aquatic habitat, abamectin will bind to sediment and dissipate from the water column generally within two weeks. However, once in sediment, it may persist with an approximate maximum half-life of 52 days.

TERRESTRIAL

The registrant responded to previous reviews on abamectin where terrestrial exposure was discussed. In these previous reviews it was concluded that while exposure on terrestrial food items was not of concern to birds, it was high enough to be hazardous to mammals.

The following theoretical values were calculated based on historical measured residue data used to generate a nomograph presented in Hoerger and Kenaga (1972)[3]. They were also presented in Merck's response on page 14 (408563-03). If abamectin is applied at 0.02 lb. ai/acre, the following residues (ppm) occur on terrestrial food items immediately after treatment.

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[2] Wislocki, Peter. Degradation of Abamectin in a Field Study Simulating Both Drift and Runoff. Report date September 23, 1986; Accession Number 400696-10

[3] Hoerger, F.C. and E.E. Kenaga. 1972. Pesticide Residues on Plants Correlation of Representative Data as a Basis for Estimation of Their Magnitude in the Environment. Environmental Quality. Academic Press, New York, I:9-28.

	Short Grass	Long Grass	Leafy Crops	Insects Forage	Seed Pods	Fruit
Maximum	4.8	2.2	2.5	1.1	0.2	0.1
Typical	2.5	1.8	0.7	0.6	0.06	0.03

At 0.025 lb. ai/acre, the following residues (ppm) were expected.

	Short Grass	Long Grass	Leafy Crops	Insects Forage	Seed Pods	Fruit
Maximum	6.0	2.7	3.0	1.5	0.3	0.15
Typical	3.1	2.3	0.9	0.8	0.07	0.04

The registrant presents several points in their attempt to negate EEB's concern for mammals. Before I continue with the assessment, these points will be addressed.

ISSUE I: On page 5 the registrant suggests that residue levels on mammalian food items will be much lower than estimated using the nomograph developed by Kenaga. Their rationale is based on results of several field residue studies in which cotton, celery, citrus and tomatoes were sampled for analysis after treatment.

EEB Response: The EEB has evaluated these results presented by the registrant. Based on that evaluation we conclude that the nomograph accurately estimates abamectin residues on food items.

For example, when cotton was treated with 0.014 and 0.018 lb. ai/acre, measured residues were 0.812 ppm and 2.1 ppm. It was not indicated whether these were averages or not and there was no indication of the variability between replicate samples. However, if these two values are assumed to be averages and modified[4] to make them equivalent to a use rate of 0.02 and 0.025 lb. ai/acre, the following values are generated.

	Use Rates from studies	
new use rate	0.014 lb/A	0.18 lb/A
0.02	1.16 ppm	2.33 ppm
0.025	1.45 ppm	2.92 ppm

This compares very closely and in fact exceeds the typical values for leafy crops estimated using the nomograph. See the tables, above.

According to the results provided, the measured residues on celery immediately after 0.02 lb. ai/acre averaged 0.1568 ppm. The

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[4] $\frac{\text{Use rate from study}}{\text{residues measured in study}} = \frac{\text{new use rate}}{\text{new residue value}}$

registrant compares these with those estimated for leafy crops. However, EEB contends that since the entire stalk of celery was collected for residue analysis, the values would more closely compare with those estimated for fruits. The large bulk of the celery stalk would result in a greater "volume to surface" ratio decreasing the concentrations of a pesticide applied to the surface. This, notwithstanding that celery does have leaves. Therefore, it is EEB's opinion that the measured residues on celery support the values estimated by the nomograph, since estimated values for fruit are 0.03 (typical) to 0.1 ppm (maximum) after an application of 0.02 lb. ai/acre.

By reviewing the Summary of Abamectin Celery Residue Data Results tables, it is clear, that while 0.1568 may be the average, many of the measured values were substantially higher than even the maximum estimation for fruit, i.e. 0.357, 0.417 and 0.540 ppm.

Measured residues on citrus fruit and tomatoes were also lower than estimated residues. However, these would also be expected to be low, since citrus and tomatoes bear relatively large fruit. These would also have a large "volume to surface" ratio.

ISSUE IA: In further discussing the use of Hoerger and Kenega to estimate residues, the registrant indicates that both the maximum and typical residue levels are highly conservative.

EEB Response: It is only the maximum residue levels that are considered "conservative". The "typical" levels are values one would expect to occur normally.

ISSUE IB: The registrant also suggests that another reason estimations based on Hoerger and Kenaga may exaggerate levels is that accumulation after multiple dosing may have been a factor.

EEB Response: Hoerger and Kenaga presented their values as occurring with just one application, even if the pesticides they were dealing with were labeled for multiple applications.

In summary, EEB disagrees with the registrant and maintains that the estimated residue levels using the nomograph presented by Kenaga are valid and will use them in risk assessments for Abamectin.

ISSUE II: They indicated they felt there was minimal concern for mammals since many of the effects were from long-term exposure (either reproductive tests or 10-day exposure studies), and abamectin is very short-lived on plant surfaces.

EEB Response: Several points require illumination:

- First, according to the 10-day study (TT# 77-717-1), the effect (mortality to one of twenty mice) at the 0.1 mg/kg level

occurred on day 3; the mortality at the 0.075 mg/kg test level occurred on day 4. While exposure occurred each day up to the time of mortality, there is no way of knowing if the effects may have been stimulated with fewer doses, but would just take a few days to manifest themselves. The EEB considers this 10-day test to be more of an acute exposure study, similar to the avian 5-day dietary study. The end result in the field, however, is likely to be reduced productivity similar to a reproductive effect;

- Second, EEB is concerned with the statement that, at least for citrus, residual control is about 90 days. How can a pesticide that virtually disappears within a few days be persistent enough to be efficacious for 90 days? Perhaps if abamectin lands on surfaces where sunlight does not penetrate foliage, or attaches to certain parts or chemicals of a plant, persistence is greater;

- Third, repeat applications are permitted, and while the registrant discusses "typical" practices of "between treatment" intervals of 60 days for citrus and 21 days for cotton, the labels do not require such intervals. To be consistent with other pesticide reviews, EEB will use label directions for this assessment; and

- Fourth, the photodegradate is also shown to be toxic (as a teratogen), to mammals at levels as low as and even lower than the parent. This precludes EEB from concluding safety because of a short halflife of parent abamectin.

ISSUE III: The registrant believes that mammalian food items are contaminated mainly thorough drift and would not be exposed to direct application.

EEB Response: The EEB considers agricultural areas such as cotton fields and citrus groves to be habitat for mammals. Therefore, as with other pesticides, we use direct application to food items as a scenario representing exposure potential. The registrant estimates that over 1.3 million acres of cotton could be treated in the San Joaquin Valley in California and about 500,000 acres in the Imperial Valley (California and Arizona). They also estimate 35,000-40,000 acres of cotton in Texas. For citrus, they further estimate a potential area of 800,000 acres in Florida, and 63,000 acres in Texas. They expect a relatively small amount used on citrus in California (<2,000 acres). Realizing that these acreages are estimations, EEB will nevertheless use them to assume that 2 to 3 million acres of land could be treated in any one year, and that any mammals dwelling within those groves or fields could be exposed to abamectin and experience the expected adverse effects.

ISSUE IV: On page 7, the data are presented to show that the mouse is the most sensitive species, (compared to the rabbit and rat).

EEB Response: While in some studies, the mouse demonstrated greater sensitivity to abamectin than other species, but in the acute oral test, the rat weanling LD50 of 1.5 mg/kg was lower than the LD50's for mice. Therefore, on an acute basis, the rat LD50 is the lowest one available.

But even more important, in order to try to ensure safety to wild mammal species that could be even more sensitive than laboratory test mammals, EEB will use the results from the testing with the most sensitive laboratory test organisms. That is, for materno-toxicity and teratogenic effects, we will use the mouse study. But for acute toxicity to young mammals, we will use the the weanling rat study.

ISSUE V: An important factor, which was touched on earlier, is the 10-day materno-toxicity study in which mortality occurred when mothers were dosed by oral gavage with 0.075 mg/kg (the NOEL was 0.05 mg/kg). Another similar study exposed the mothers for 10 days through the diet. The NOEL was 0.1 mg/kg. The registrant would like to use the 0.1 mg/kg NOEL rather than the 0.05 mg/kg, since mammals would likely ingest abamectin via their diet, rather than through oral gavage.

EEB Response: With just two tests on which to base assumptions, EEB (conservatively) will use the lower value because it is not clear that the dietary study actually represents a difference, rather than just normal variation between different studies.

The result from the 10-day gavage study is used later in the review to develop NOEL's for materno-toxicity.

ISSUE VI: The registrant suggests that the delta 8,9 isomer, a photodegradate is of minor toxicological concern.

EEB Response: Granted, if a degradate makes up only 10-20% of the combined residues, we normally have minimal concern. However, in a case where the parent exhibits a serious toxicological characteristic which is echoed by the degradate, it becomes more important. Especially in this case where photodegradation of the parent is presented as a factor mitigating hazard to mammals and the degradate poses the same hazard. The fact that the registrant claims 90 days efficacy again seems to be relevant. It may be that the photodegradates play an important role in the overall toxicity of abamectin. This precludes EEB from concluding safety to mammals.

ISSUE VII: The registrant points to use of Ivermectin (a closely related compound) on mammals as an indication of safety to nontarget mammals.

EEB Response: In a literature search, EEB discovered references of adverse toxicological responses in horses, dogs and tortoises treated with Ivermectin. In one study, a dog was treated with Ivermectin for suspected endoparasitism. Within 2 hours the dog had hind limb ataxia and was semicomatose within 20 hours[5]. When collies were dosed orally with 100 micrograms/Kg (0.1 mg/Kg), 3 of 14 developed mild toxic symptoms; then a second dose in the same dogs at 200 micrograms/Kg (0.2 mg/Kg) caused severe toxicosis (seizure-like activity, nonresponsiveness, and coma) in 7 of the 14[6]. Horses (366 of 3316 treated) developed adverse reactions to Ivermectin treatment; one death was reported[7]. The red-footed tortoises (Geochelone carbonaria) dosed at 0.4 mg/Kg experienced extreme paresis and flaccid paralysis; The leopard tortoise (Geochelone pardalis) consistently developed mild paralysis with a dosage of 0.025 mg/kg and death even occurred at 0.3 mg/Kg[8].

Granted, these reports of toxic response are with Ivermectin, and cannot be included in the risk assessment on avermectin. However, they certainly negate the registrants claim that Ivermectin is used safely with domestic mammals and other animals.

Mammal Assessment

Using an acute oral LD50 of 10 mg/Kg for adult rats the following 1-day adult LC50 values (ppm) were calculated[9] for selected mammals. The weanling 1 day LC50 values were based on a 1.5 mg/kg LD50 for weanling rats. The third column in the table is the extrapolated reproductive NOEL's (ppm) based on the rat 1-

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[5] Houston, D.M., J. Parent and K.J Matushek. Ivermectin Toxicosis in a Dog. J Am Vet Med Assoc, Vol 191(1), 1987, pp78-80

[6] Paul A.J. et al. Clinical Observations in collies Given Ivermectin Orally. Am J Vet Res, Vol 48(4), 1987, p684-5.

[7] Karns, P.A. and D.G. Luther. A Survey of Adverse Effects Associated with Ivermectin use in Louisiana Horses. J Am Vet Med Assoc, Vol 185(7), 1984, p782-3

[8] Teare, J.A and M. Bush. Toxicity and Efficacy of Ivermectin in Chelonians. J Am Vet Med Assoc Vol 183(11), 1983, p1195-7.

[9] LC50 (ppm) = LD50 X wt (g) / consumption in one day (g).

generation reproductive test[10]. The fourth column is the extrapolated materno-toxicity NOEL's in ppm based on the mouse 10-day test (TT# 77-717-1)[11]. The weight and food consumption data are from Davis and Golly (1963)[12].

Grazing Herbivores	1 day LC50 (ppm) adult	weanling/young	Rep. LEL (ppm)	Materno-Toxic NOEL ppm
Meadow vole	16	2.5	0.16	0.08
Cotton rat	32	4.8	0.32	0.16
Deer	412	61.4	4.1	2.06
Granivores				
Red squirrel	142	21.3	1.4	0.7
Omnivores				
Deer mouse	51	7.7	0.5	0.26
Marsh rice rat	218	32.6	2.2	1.09
Raccoon	470	70.8	4.7	2.36
Insectivores				
Least shrew	9	1.4	0.09	0.05
Carnivore				
Least weasel	40	6	0.4	0.2

Estimated residues are presented again for ease of comparison. If abamectin is applied at 0.02 lb. ai/acre, the following residues (ppm) occur on terrestrial food items immediately after treatment.

	Short Grass	Long Grass	Leafy Crops	Insects Forage	Seed Pods	Fruit
Maximum	4.8	2.2	2.5	1.1	0.2	0.1
Typical	2.5	1.8	0.7	0.6	0.06	0.03

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[10] Reproductive NOEL (ppm) = rat NOEL X wt (g) / consumption in one day (g). NOEL=0.1 mg/kg/day.

[11] $\text{10-day materno-toxicity NOEL (ppm)} = \frac{\text{mouse NOEL (mg/kg) X wt (g)}}{\text{consumption in one day (g)}}$

[12] Davis, D.E. and F.B. Golly. 1963. Principles of Mammalogy. Reinhold Publ. Corp. NY.

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At 0.025 lb. ai/acre, the following residues (ppm) were expected.

	Short Grass	Long Grass	Leafy Crops	Insects Forage	Seed Pods	Fruit
Maximum	6.0	2.7	3.0	1.5	0.3	0.15
Typical	3.1	2.3	0.9	0.8	0.07	0.04

The extrapolated adult 1-day LC50's are not exceeded by the estimated residues on terrestrial food items. The estimated residues on short and long grass and leafy crops equal or exceed the 1-day LC50 for weanling meadow voles. Therefore acute effects may occur to certain young mammals.

Typical residues on most vegetation exceed the materno-toxicity NOEL for small herbivores and residues on insects exceed this level for insectivores at both application levels. This level is expected to result in mortality to pregnant mothers feeding on contaminated materials.

The extrapolated reproductive NOEL's are exceeded by typical residues on food items for grazing herbivores, omnivores, and insectivores of small size. Granivores and carnivores would not likely ingest food with residues greater than their reproductive NOEL. While abamectin may degrade rather rapidly on plant surfaces as evidenced by submitted residue data, it is applied three times per season on citrus and cotton (between treatment interval not specified). Since the label does not specify between treatment intervals, chronic exposure and hazard are assumed to be possible. In any case, the materno-mortality, while not an effect on reproductive physiology per se, is still, in essence, a reproductive effect since the dead mother cannot produce young. And as was stated earlier, this effect could occur with a very short exposure (1 to 4 days).

Based on this, it is likely that the use of Abamectin at 0.02 and 0.025 lb. ai/A would cause acute effects to young grazers; materno-toxicity to herbivores and insectivores, and chronic effects to certain grazing herbivores, omnivores and insectivores.

Even though avermectin is supposed to photodegrade rather rapidly, there are some issues negating this mitigating factor.

- The photodegradate is considered more toxic to mammals than the parent, (delta 8,9-isomer causes mammalian teratogenic effects at lower levels than the parent);

- Multiple applications may result in repeated exposures to some mammals during their gestation period, and a greater risk

of exposure during at least one gestation period for smaller mammals which reproduce more than once per year; and

- Through some mechanism, avermectin exhibits residual efficacy for up to 90 days. Extended efficacy is claimed by the registrant for both citrus and fire ant control. The extended fire ant control is explained by the fact that the ants carry the parent underground where it is no longer exposed to light. This supports the contention that reducing exposure to light will extend the halflife. Some residues are likely to be deposited to surfaces not exposed to light, thereby reducing substantially the photodegradation rate. It is also possible that abamectin residues chemically react with some plant parts (possibly oils), thus inhibiting photodegradation and extending their halflife.

- The materno-mortality exhibited in mice occurred within 3 to 4 days, making it more of an acute effect from short-term exposure than a chronic effect.

Mammal Summary

The estimated residues on vegetation will be used to assess hazard to mammals. Based on a comparison of these levels, the use of abamectin on citrus and cotton at 0.02 to 0.025 lb. ai/acre is considered acutely hazardous to young mammals dwelling in, or foraging within, treated areas. It is also expected to exhibit materno-toxicity to pregnant mammals feeding on treated food items. Chronic hazard will increase as the "between treatment" interval shortens. Mammalian field testing is required to address this concern.

Bird Assessment

These residues do not exceed the lowest avian dietary LC50 of 383 ppm nor the avian reproductive NOEL of 12 ppm. Therefore, no acute or chronic hazard to birds is expected.

AQUATIC

The registrant has responded to previous reviews with a Summary of Abamectin Aquatic Toxicology and Environmental Fate Studies, Accession No: 408563-02. Several issues need to be addressed in this summary.

ISSUE I: The registrant indicates that chronic exposure does not enhance toxicity.

EEB Response: Chronic exposure increases the capacity to kill organisms, and since dead organisms cannot reproduce, this is, in essence reproductive effect.

ISSUE II: The field study submitted earlier (Wislocki, 1986), was used to show exposure levels in the aquatic habitat.

EEB Response: While EEB has reviewed this study and has found it quite useful, it did not actually measure runoff or drift. It assumed a certain amount of the applied pesticide would either transport via runoff or drift and that amount was loaded into the small ponds (actually tanks containing sediment and water). It provides useful information on the fate of abamectin and will be used extensively in the following risk assessment. The actual measured concentrations will not be used to assess hazard or conclude safety.

Further, the registrant points out on page 4 that concentrations from runoff in the water column are below levels of concern and then they discuss the rapid degradation of the treated soil that was used in the aged dosing regime. The discussion does not point out that concentrations in sediment persisted for up to 52 days.

ISSUE III: On page 5, the registrant refers to the 6-foot depth as required for viability.

EEB Response: The 6-foot depth is used in many of our estimations of aquatic exposure as being typical. However, it must be recognized that many viable aquatic habitats are significantly shallower than 6 feet.

ISSUE IV: The registrant indicates that because of the lack of effect on the sexual reproduction of shrimp and the low bioconcentration potential, that the fish full life cycle study is not required.

EEB Response: Abamectin effects mammal reproduction at extremely low rates. This in conjunction with persistence in the water column (see discussion below) and sediment and multiple applications is justification for requesting the fish full life cycle study. Furthermore, concentrations in aquatic habitat from the cotton use are estimated to be >0.1 the NOEL derived from the fish early-life stage test. The study is required.

ISSUE V: Page 8 indicates that estuaries are continually flushed with freshwater because of tidal mixing.

EEB Response: While most major estuaries do receive fresh water continually as a river or stream flushes the system, smaller bays and inlets that do not form the mouth of a stream would not be flushed. In these cases, the tidal movement would simply serve to shift the contents of the water body back and forth over the same aquatic habitat. Furthermore, these estuarine wetlands are usually much shallower than 6 feet and are essential in the productivity of the aquatic ecosystem. Because they are shallower,

concentrations from drift and runoff in estuaries would not be diluted as much and could be more hazardous to estuarine organisms.

ISSUE VI: On page 7, the registrant indicates that the end point of the mesocosm is to measure effects to fish, and that since direct effects are unlikely, a mesocosm is not necessary.

EEB Response:

First: The endpoint of the mesocosm, or any multiple pond biological field study is not necessarily fish productivity. EEB is concerned with aquatic invertebrates both in the water column and in the sediment.

Second: If invertebrate populations are reduced, it could have effects on fish productivity, due to reduced or altered food supply.

Third: Until the fish full life cycle study has been completed, it is not possible to determine if concentrations in the water and sediment are chronically hazardous to fish.

Aquatic Assessment

The following toxicity information will be used to assess impact to aquatic organisms.

Acute Values

Rainbow trout	LC50=3.2 ppb
Daphnia magna	LC50 0.22-0.34 ppb
Mysid shrimp	LC50= 0.2 ppb (15% to 20% mortality at 4.3 ppt)
Oyster embryo-larvae	LC50= 430 ppb

Chronic Values

Daphnia magna	life-cycle	MATC >0.03<0.09 ppb (all dead by day 5 at 0.09 ppb)
Rainbow trout	early life stage	MATC >0.52<0.96 ppb
Mysid Shrimp	Life-cycle	MATC >0.0035<0.0093 ppb

Concern Levels Summary

Abamectin is expected to have an acute effect on fish at 1.6 ppb (1/2 LC50) and a chronic effect on fish if persistent (>4 day) residues exceed the NOEL of 0.52 ppb.

Aquatic invertebrates (shrimp) will experience some mortality if concentrations exceed 0.0043 ppb (4.3 ppt). If concentrations equal or exceed 0.09 ppb (90 ppt) for 5 days, all invertebrates (Daphnia magna) would be expected to die. Tests, thus far, have

not shown that reproduction physiology of aquatic invertebrates is affected by abamectin at levels below those which cause mortality. However, extending exposure does enhance lethal effects.

Exposure and Hazard

Abamectin has low solubility (7.8 ppb), and high octanol water partition coefficient (9.9 X 10_3).

Exposure to aquatic organisms will be based on two different things. The first is computer modeling using SWRRB and EXAMS. Second, the registrant has performed what was referred to as a simulated runoff and drift study in which concentrations of Abamectin were measured in water and sediment. The purpose of the study was to provide information to show environmental behavior and potential exposure concentrations once a certain amount of abamectin reaches the aquatic habitat. The dose levels used in the study were exaggerated to facilitate increased sensitivity of chemical analysis methods for measuring Abamectin in water and sediment. Therefore, the measured concentration values will be used to characterize the fate of Abamectin, rather than as actual exposure concentrations. Review of the study can be found in the 9-14-87 EEB review on Cotton.

Exposure Concentrations Estimated for Cotton 0.02 lb ai/acre

Exposure Due to Drift:
Cotton use is assumed to exclude aerial appl., drift assumed to be negligible.

Exposure Due to Runoff: A SWRRB model was run to determine the amount of abamectin likely to transport via runoff. The assumptions included an average year with regards to rainfall, an application rate of 0.02 lb. ai/acre with three applications at 14 day intervals during June-July. Then, using the loading from SWRRB, an EXAMS model was used to determine concentrations in water and sediment of a pond and a stream flowing from the pond. The maximum concentration in pond water reached 0.6 ppb, with a maximum concentration in the receiving stream (100 meters long by 3 meters wide and 0.5 meters deep) of 0.4 ppb. Concentrations in sediment reached 2.8 ppb and 4.3 ppb in the pond and stream, respectively.

	EEC ppb	
	pond	stream
water	0.606	0.4
sediment	2.828	4.350

ACUTE DISCUSSION

These concentrations exceed the Daphnia magna acute EC50 (0.22 - 0.34 ppb) and the shrimp LC50 (0.02 ppb). The concentrations in the water column do not exceed the fish acute concern level of 1.6

ppb (1/2 LC50 of 3.2 ppb), nor do the concentrations in the sediment exceed 1/2 the oyster EC50 (430 ppb/2=215 ppb). Acutely, the expected concentrations from use of abamectin on cotton are not expected to affect fish or mollusks. However, acute effects to aquatic and estuarine invertebrates would be expected.

Concentrations in the sediment are higher than the fish LC50 and substantially higher than invertebrate concern concentrations. It is not known how "available" or toxic these bound residues are; however, EEB does not assume they are unavailable. Biological aquatic field testing is required to determine the impact of concentrations in the water column and in the sediment.

CHRONIC DISCUSSION

Both the EXAMS model and the study provided by the registrant provided information on the persistence and fate of abamectin once it reaches the water.

Persistence and Fate from EXAMS

After the initial high concentration of 0.6 ppb, the following table shows the decline over several weeks.

	Concentrations (ppb)			
	pond		stream	
Julian date	water	sediment	water	sediment
170 Application				
188	0.61	0.61	0.28	1.3
189	0.58	0.80	0.40	1.7
190 Appl.	0.55	0.97	0.40	2.1
191	0.53	1.14	0.38	2.5
192	0.50	1.28	0.36	2.8
193	0.48	1.42	0.35	3.1
194	0.46	1.55	0.34	3.3
195	0.44	1.67	0.32	3.5
196	0.42	1.77	0.31	3.7
197	0.40	1.87	0.29	3.8
198	0.38	1.96	0.28	4.0
199	0.37	2.04	0.27	4.1
200	0.35	2.11	0.26	4.2
201	0.34	2.18	0.25	4.2
202	0.32	2.24	0.24	4.3
204 Appl.				
205	0.28	2.39	0.21	4.3
210	0.33	2.5	0.17	4.2
215	0.27	2.7	0.20	4.3
220	0.22	2.82	0.17	4.2
225	0.18	2.82	0.14	3.9
230	0.15	2.76	0.11	3.6

Based on these estimates, the concentration in the water column, after 40 days, in both the pond and stream exceed the Daphnia magna life cycle NOEL and LOEL of 0.03 and 0.09 ppb, and the shrimp life cycle NOEL and LOEL of 0.0035 and 0.0093 ppb, respectively. These estimations even exceed the fish NOEL of 0.52 ppb for four days and 1/10 the fish NOEL (0.052 ppb) for 40 days.

However, in the study reviewed in 9-14-87 residues in water tended to decline more rapidly than the exams model suggested. Both "rates of dissipation" will be used, the EXAMS model will serve as the more conservative estimate. This will provide a range of exposure estimates on which to base an assessment.

The following tables show how the actual measured concentrations from the 1986 study are used to estimate long-term exposure levels using the highest EXAMS estimate as the basis.

TABLE 1 Expected Concentrations Based on Values Described as Immediate Runoff Simulation (Table 6 in study report)

	Day Following Dosing (all values in ppb)						
Replicate	0	1	2	4	8	15	31
A							
Measured[13]	0.2	ND[14]	0.1	0.52	0.1	0.11	ND
Percent[15]	38		19	100	19	21	
EEC[16] (ppb)	0.23		0.12	0.606	0.12	0.13	
B							
Measured	0.25	0.43	0.26	0.46	0.24	0.35	0.1
Percent	54	93	56	100	52	76	22
EEC (ppb)	0.33	0.57	0.34	0.606	0.32	0.46	0.13
C							
Measured	3.52	0.78	3.52	3.52	3.68	1.28	0.24
Percent	95	22	95	95	100	35	6.5
EEC (ppb)	0.58	0.13	0.58	0.58	0.606	0.21	0.039

[13] Actual measurements from field study in ppb.

[14] ND= no residues detected in field study.

[15] Using the highest value measured in the field study, each lower value is transformed to a percent of that value.

[16] Using the percent in the line above, the initial value of 0.606 ppb is reduced.

Based on this, it is estimated that concentrations in water will exceed chronic concern levels for fish (0.52 ppb) for four days. Concentrations will exceed the Daphnia magna life cycle NOEL and LOEL of 0.03 and 0.09 ppb, and the shrimp life cycle NOEL and LOEL of 0.0035 and 0.0093 ppb, respectively, for 31 days.

Exposure Concentrations Estimated for Citrus 0.025 lb ai/acre

Exposure Due to Drift (5%):
(Mist blowers are assumed to result in drift equal to aerial application).

The following table is based on the residues measured in the "drift simulation" tanks in the 1986 field study discussed earlier.

	Concentration (ppb) On Day					
REPLICATE	0	1	2	4	8	15
A Measured (ppb)[17]	1.03	0.24	ND[18]	ND	ND	0.11
Percent of max.[19]	100%	23.3%				10%
B measured (ppb)	12.5	0.85	0.17	0.1	ND	ND
Percent of maximum	100%	6.8%	1.4%	0.9%		
C Measured (ppb)	13.7	1.05	0.73	0.77	0.29	0.10
Percent of mximum	100%	7.6%	5.3%	5.6%	2.1%	0.7%
RATE lb ai/acre						
0.025 EEC(ppb)[20] min	0.076	0.005	0.001	<.001	<.001	<.001
		0.018	0.004	0.004		0.008

Exposure Due to Runoff: Based on previous experience, surface runoff from citrus is expected to be negligible. In citrus growing areas, where soil has a high sand content, transport of pesticides via groundwater is possible. However, abamectin does not tend to move in groundwater, so this route of exposure is unlikely. Concentrations in adjacent aquatic habitat due to runoff are expected to be less than concern levels for both acute and chronic effects.

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[17] Actual measurements from field study in ppb.

[18] ND = no residues detected in field study

[19] Using the highest value measured in the field study, each lower value is transformed to a percent of the highest value.

[20] Based on an assumed 5% drift into 6 feet of water and using the percent(s) in the replicates above to determine concentrations over time.

Immediate concentrations due to drift from treatment of citrus at 0.025 lb: ai/acre exceed the shrimp LC50's of 0.020 to 0.033 ppb. These estimations do not exceed the Daphnia magna EC50 of 0.22 to 0.34 ppb. However, they exceed the Daphnia magna life cycle NOEL and LOEL of 0.03 and 0.09 ppb, and the shrimp life cycle NOEL and LOEL of 0.0035 and 0.0093 ppb. Even though the levels drop below acute and chronic concern levels within 2 days, multiple applications are likely to result in repeated exposure and chronic effects.

It is not known if the concentrations in sediment are available and toxic.

Aquatic Summary

A comparison of available data with exposure estimates based on modeling and an aquatic fate study indicate that the concentrations in water from both the cotton and citrus use would be expected to result in adverse acute or chronic effects to aquatic organisms. The fish full life cycle study is still required and may result in a concern for fish reproductive effects. Field testing is required (see discussion in conclusions). The fish full life cycle test is required in addition to the field study to assist in the interpretation of the field study results. The results of the fish full life cycle study would be beneficial in the design of the aquatic field study.

101.3 Endangered Species Considerations

The triggers that will be used to determine whether endangered species may be affected by the use of Abamectin are presented below.

	Acute Trigger	Chronic Trigger
Bird	38.3 ppm (1/10 LC50)	>12 ppm
Mammal	0.05 mg/kg[21]	>0.1 mg/kg
Reptile		
Terrestrial Amphibians		
Terrestrial invertebrates: Effects assumed if exposure occurs		
Fish	0.16 ppb (1/20 LC50)	>0.52 ppb
Aquatic Invertebrate	0.011 ppb (1/20 LC50)	>0.0035 ppb (shrimp)
Mollusk	21.5 ppb (1/20 EC50)	

Cotton

Exposure Concentrations Estimated for Cotton 0.02 lb ai/acre

Estimated residues are presented again for ease of comparison. If abamectin is applied at 0.02 lb. ai/acre, the following residues (ppm) occur on terrestrial food items immediately after treatment.

	Short Grass	Long Grass	Leafy Crops	Insects Forage	Seed Pods	Fruit
Maximum	4.8	2.2	2.5	1.1	0.2	0.1
Typical	2.5	1.8	0.7	0.6	0.06	0.03

Exposure Due to Drift:
Cotton use is assumed to exclude aerial appl., drift assumed to be negligible.

Aquatic Exposure Due to Runoff: A SWRRB model was run to determine the amount of abamectin likely to transport via runoff. See the discussion above for an explanation of assumptions and calculations.

	EEC ppb	
	pond	stream
water	0.606	0.4
sediment	2.828	4.350

[21] Materno-toxicity NOEL

Estimated exposure concentrations do not exceed the concern levels for birds or mollusks. Based on available information, endangered birds and mollusks are not expected to be affected by the use of Abamectin on cotton.

Endangered mammals, reptiles, terrestrial amphibians, invertebrates and fish may be impacted by the use of abamectin on cotton. The EEB is in the process of updating the cotton cluster. The results of the FWS biological opinion on that cluster will be applicable to the registration of abamectin on cotton.

Citrus

Exposure Concentrations Estimated for Citrus 0.025 lb ai/acre

At 0.025 lb. ai/acre, the following residues (ppm) were expected.

	Short Grass	Long Grass	Leafy Crops	Insects Forage	Seed Pods	Fruit
Maximum	6.0	2.7	3.0	1.5	0.3	0.15
Typical	3.1	2.3	0.9	0.8	0.07	0.04

Exposure Due to Drift (5%):
(Mist blowers are assumed to result in drift equal to aerial application)

The following table is based on the residues measured in the "drift simulation" tanks in the 1986 field study discussed earlier.

	Concentrations (ppb) On Day					
REPLICATE	0	1	2	4	8	15
A [Measured (ppb)]	1.03	0.24	ND	ND	ND	0.11
Percent of maximum	100%	23.3%				10%
B [Measured (ppb)]	12.5	0.85	0.17	0.1	ND	ND
Percent of maximum	100%	6.8%	1.4%	0.9%		
C [Measured (ppb)]	13.7	1.05	0.73	0.77	0.29	0.10
Percent of mximum	100%	7.6%	5.3%	5.6%	2.1%	0.7%
RATE lb ai/acre						
0.025 EEC(ppb)[22] min	0.076	0.005	0.001	<.001	<.001	<.001
		0.018	0.004	0.004		0.008

[22] Based on an assumed 5% drift into 6 feet of water and using the percent(s) in the replicates above to determine concentrations over time.

Exposure Due to Runoff: Based on previous experience, runoff from citrus is expected to be negligible. Abamectin does not tend to move in groundwater. Concentrations in adjacent aquatic habitat due to runoff are expected to be less than concern concentrations for both acute and chronic effects.

Estimated exposure concentrations do not exceed the concern levels for birds or mollusks. Based on available information, endangered birds and mollusks are not expected to be affected by the use of Abamectin on citrus.

Endangered plants are not considered to be at risk because of lack of exposure potential. That is, endangered plants do not occur near citrus or cotton growing areas.

Certain endangered mammals, reptiles, terrestrial amphibians, and invertebrates may be impacted by the use of abamectin on citrus. Fish may be impacted indirectly through loss of food supply. Chronic effects to fish may be expected depending on the results of the fish full life cycle test. Citrus is grown in Arizona, California, Florida, and Texas. The following table identifies the endangered species (not including birds or plants) that occur in counties where citrus is grown.

Species	County	State
Sonoran pronghorn	Yuma	AZ
Gila topminnow	Maricopa	AZ
Stephen's kangaroo rat	Riverside, San Diego	CA
Fresno kangaroo rat	Fresno	CA
Giant kangaroo rat	Fresno, Kern, San Luis Obispo, Santa Barbara, Tulare	CA
Tipton Kangaroo rat	Fresno, Kern, Tulare	CA
Morro Bay kangaroo rat	San Luis Obispo	CA
Valley Elderberry lnghrn beetle	Glenn	CA
Kern primrose sphinx moth	Kern	CA
Coachella Valley fringe-toed lizard	Riverside	CA
Bluntnose leopard lizard	Fresno, Kern, Madera, San Luis Obispo, Tulare	CA
Island night lizard	Santa Barbara, Ventura	CA
Desert slender salamander	Riverside	CA
Bonytail chub	San Bernardino	CA
Mohave tui chub	San Bernardino	CA
Unarmored threespine stckleback	San Bernardino, Santa Barbara	CA

Species	County	State
Little Kern golden trout	Tulare	CA
Paiute cutthroat trout	Madera	CA
Lahontan cutthroat trout	Madera	CA
Desert pupfish	Imperial, Riverside	CA
Southeastern beach mouse	Brevard, Indian River, St. Lucie, Volusia	FL
Sand skink	Highlands, Lake, Marion Orange, Polk	FL
Blue-tailed mole skink	Polk	FL

Discussion

The bioaccumulation factor for abamectin is not particularly high, therefore, exposure to secondary consumers such as the San Joaquin kit fix, Florida panther, American alligator, the Eastern indigo snake, the Atlantic Salt Marsh snake, and the ocelot is unlikely.

The EEB must consult with the US Fish and Wildlife Service to determine which species may be jeopardized by the use of Abamectin on Citrus and what prudent and reasonable alternatives could reduce impact to endangered species. This consultation will be initiated when the fish full life cycle test has been completed.

101.4 Adequacy of Data

The available data were adequate to complete a risk assessment that results in a presumption of hazard to aquatic organisms and mammals. The only additional laboratory study required is a fish full life cycle test. This test is required since Avermectin is applied repeatedly and causes reproductive (teratogenic) effects to mammals at very low levels. The effects to mammals suggest avermectin may affect reproduction of other organisms. Furthermore, based on our estimations of exposure, chronic exposure exceeds 1/10 the fish early life stage NOEL of 0.052 ppb.

Aquatic field testing is required because of expected acute effects to aquatic invertebrates and because long term exposure exceeds chronic levels of concern for aquatic organisms.

Terrestrial field testing is required to either quantify the effects of Avermectin on wild mammals, or to show that effects observed in the laboratory tests do not occur in the field.

101.5 Adequacy of Labeling

Minor changes in the labeling are required. It should read:

"This pesticide is toxic to fish and wildlife. Do not apply directly to water or wetlands (swamps, bogs, marshes and potholes). Do not contaminate water when disposing of equipment wash water or rinsate.

This product is highly toxic to bees exposed to direct treatment or residues on blooming crops or weeds. Do not apply this product or allow drift to blooming crops or weeds if bees are visiting the treatment area."

102 Restricted Use Categorization

The use of Abamectin on Citrus and Cotton exceeds restricted use criteria.

			Exposure[23]	
Organism	LC50	Trigger	Cotton 0.02 lb.	Citrus 0.025 lb.
Mammals				
Herbivores	2.5 ppm/5 =	0.5 ppm	4.8 ppm*	6.0 ppm*
Insectivores	1.4 ppm/5 =	0.28 ppm	1.1 ppm*	1.5 ppm*
Birds	383 ppm/5 =	76.6 ppm	4.8 ppm	6.0 ppm
Fish	3.2 ppb/10=	0.32 ppb	0.6 ppb*	0.091 ppm
Aqu. Inv.				
D. magna	0.22 ppb/10=	0.022ppb	0.6 ppb*	0.091 ppm*
Shrimp	0.02 ppb/10=	0.002ppb	0.6 ppb*	0.091 ppm*

* Exposure exceeds restricted use criteria.

Abamectin use on cotton exceeds restricted use criteria because of hazard to mammals, fish, and aquatic invertebrates. Abamectin use on citrus exceeds restricted use criteria because of hazard to mammals and aquatic invertebrates.

103 Conclusions

The EEB has reviewed the proposed use of Avermectin on Citrus and Cotton. Based on laboratory data abamectin is extremely toxic

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[23] Taken from discussions of exposure under hazard assessment.

to aquatic invertebrates and mammals. When compared to expected exposure concentrations, these uses are likely to result in acute and chronic adverse effects to mammals, and aquatic and estuarine invertebrates. Fish are not expected to be impacted acutely; however, until the fish full life cycle has been provided, a final conclusion cannot be made regarding potential chronic effects to fish. Fish productivity may be affected through loss of their food supply.

NOTE TO PM: This assessment assumed that cotton would only be treated by ground equipment. If actual use involves aerial application, it would be necessary to modify this risk assessment.

Testing Required

1. Fish full life cycle study using technical Abamectin.

The following field test requirements are established on a presumption of risk and hazard to mammals and aquatic organisms based on a comparison of estimations of exposure levels to laboratory results. These data are not simply more guideline studies to complete the data base.

2. Aquatic Biological Field Testing: Study must include multiple pond/multiple dose regime to bracket exposure concentrations expected in natural habitat. A mesocosm type study with artificial ponds is acceptable. In any case, the study must address effects to aquatic invertebrates and fish productivity. It is recommended that the registrant use sufficient dose levels and repeated doses to cover as many proposed uses as possible. The registrant should submit a protocol for EEB to evaluate. The protocol must, among other things, identify the specific amounts and methods of dosing.

3. Mammal Field Testing: This study must incorporate observation and measurement of small mammal productivity and survival at multiple fields and crop sites where abamectin is proposed for use. A carcass search type study is not sufficient. It will likely involve the use of replicated control and treatment plots in which survival of adults and young is measured. The study may involve studying populations of marked mammals likely to ingest material contaminated with abamectin when it is used according to label directions. It would be necessary to perform preliminary laboratory testing with the mammals likely to be studied in the field to determine their sensitivity. Studies must be conducted in both citrus and cotton growing areas. The registrant must submit protocol for evaluation. The protocol should, among other things, identify the locations where the testing will be done. As an alternative, the protocol should define in great detail what characteristics the sites must have. It should also indicate what

species will be studied and why these are appropriate. (For example, of the mammals living in proposed treatment areas, the chosen species are the most sensitive, and are the most likely to consume contaminated material.) The EEB is willing to meet with the registrant to discuss the conduct of these studies. It would be more productive if protocols, or at least detailed proposals of study, were submitted with sufficient lead time for review by EEB before the meeting.

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Norman J. Cook 4.11.89
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Accession No. 408563-04

DATA EVALUATION RECORD

1. **CHEMICAL:** ^{3}H-Avermectin B_1
Shaughnessey No. 122804

2. **TEST MATERIAL:** ^{3}H-Avermectin B_1; Lot #L-676,863-164L010; ^{3}H-avermectin B_1 was a 7.95:1 mixture of avermectin B_{1a} and avermectin B_{1b}, with the tritium label (at the 5-position) present only in the avermectin B_{1a} fraction.

3. **STUDY TYPE:** Estuarine Organism 96-hour Flow-Through Toxicity Test. Species Tested: Mysid Shrimp (Mysidopsis bahia)

4. **CITATION:** Suprenant D.C. (1988) Acute Toxicity of ^{3}H-Avermectin B_1 to Mysid Shrimp (Mysidopsis bahia) Under Flow-Through Conditions. Prepared by Springborn Life Sciences, Wareham, Massachusetts. Submitted by Merck and Company, Inc., Rahway, New Jersey. Accession No. 408563-04.

5. **REVIEWED BY:**

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Signature:

Date:

6. **APPROVED BY:**

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Staff Toxicologist
KBN Engineering and
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Signature:

Date: Daniel Rieder 4-14-89

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature:

Date: Henry T. Craven

7. **CONCLUSIONS:** This study appears scientifically sound and fulfills the Guideline requirements for a 96-hour flow-through acute toxicity study for estuarine and marine shrimp. The 96-hour LC50 based upon mean measured concentrations of ^{3}H-Avermectin B_1 to the mysid (Mysidopsis bahia) was 22 ng/L. Therefore, ^{3}H-Avermectin B_1 is classified as very highly toxic to the mysid.

8. **RECOMMENDATIONS:** N/A

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: The mysids used in this toxicity test were cultured and acclimated at the testing facility. Prior to testing, mysids were maintained in natural filtered seawater under recirculating conditions. Mysids were fed brine shrimp nauplii two times daily and Hatchfry Encapsulon^{R} three times weekly. The mysid culture area received a regulated photoperiod of 16-hours light and 8-hours darkness. Commercial aquarium heaters were used to maintain the culture solution temperatures at 25 $\pm$ 1^{o}C. The maximum test organism biomass was < 3 mg/L at any given time during the test.

B. Test System: The test was conducted using an exposure system consisting of a modified Mount and Brungs (1967) proportional diluter, a temperature controlled water bath, and a set of 14 test aquaria. The test system was designed to provide five concentrations of test material, a dilution water (seawater) control and solvent control. The solvent control solution contained the maximum amount of acetone present in any test concentration (2.2 uL/L). Each glass test aquarium measured 39 x 20 x 25 centimeters (cm) with a self-starting siphon attached to a system drain. Two mysid retention chambers, constructed from glass petri dishes and nylon screen (363-um mesh size opening), were positioned in each aquarium. This system allowed the aquarium volume to fluctuate between 3.1 and 7.0 L. The flow rate of exposure solutions to each test aquaria was equivalent to 7-volume additions per 24 hours. Test aquaria were impartially positioned in a water bath containing circulating water heated by immersion coil heaters and regulated by a mercury column thermoregulator designed to maintain the test solution temperature at 25 $\pm$ 1^{o}C.

C. Dosage: 96-hour acute flow-through test.

D. Design: Selection of ^{3}H-Avermectin B_1 concentrations for the 96-hour acute toxicity test with mysid shrimp was based on preliminary exposures of M. bahia to ^{3}H-Avermectin B_1. The test was initiated when 20 ($\leq$ 24-hours old) mysid shrimp were randomly distributed to each concentration or control (10 mysids per replicate). A control, solvent control and nominal ^{3}H-Avermectin B_1 concentrations of 4.5, 6.9, 11, 16, and 25 ng/L were maintained. All concentrations were observed once every

24 hours for mortality and abnormal effects. The water quality parameters (dissolved oxygen, pH, salinity, and temperature) were measured and recorded daily for each replicate of the control solutions and each treatment level. Test solution temperature was also continuously monitored in one replicate of the solvent control solution throughout the study. Analytical determination of ^{3}H-Avermectin B_1 was performed on all treatment levels at 0 and 96 hours using radiometric analysis.

E. Statistics: The mean measured concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate the median lethal concentrations (LC50) and 95% confidence intervals for each 24-hour interval of the exposure period. LC50 values were empirically estimated as being greater than the highest concentration tested when no test concentrations caused 50% or more mortalities. If at least one test concentration caused mortality of greater than or equal to 50 % of the test population, then a computer program (Stephan, 1977, 1982) was used to calculate the LC50 values and 95% confidence intervals.

12. REPORTED RESULTS: Analytical determination of test concentrations resulted in mean measured concentrations of 4.2, 7.7, 10, 16, and 29 ng/L. The mean measured concentrations were 91 to 116% of the nominal concentrations. "The mean measured test concentrations, the corresponding mortalities and the observations made during the 96-hour test are presented in Table 3 (attached). After 96 hours of exposure mortality was observed among 80, 15, and 15% of the mysids at the three highest mean measured test concentrations (29, 16 and 10 ng/L ^{3}H-Avermectin B_1, respectively). Mortality of $\leq$ 5% was observed among mysids exposed to the remaining treatment levels (7.7 and 4.2 ng/L ^{3}H-Avermectin B_1). Based on these data, the 96-hour LC50 (95% confidence interval) for ^{3}H-Avermectin B_1 and mysids was calculated to be 22 (16 - 29) ng/L. Mortality among control organisms during the study was $\leq$ 10%. Based on criteria established by U.S. EPA (1985), ^{3}H-Avermectin B_1 would be classified as very highly toxic to mysid shrimp." The 24-, 48-, 72-, and 96-hour LC50 values for mysids exposed to ^{3}H-Avermectin B_1 was estimated to be >29, >29, 33, and 22 ng/L based on mean measured concentrations. The no observed effect concentration (NOEC) at 96-hours was 7.7 ng/L based on mean measured concentrations.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 24-, 48-, 72-, and 96-hour LC50 values for mysids exposed to ^{3}H-Avermectin B_1 was estimated to be >29, >29, 33, and 22 ng/L based on mean measured concentrations. The no observed effect concentration (NOEC) at 96-hours was 7.7

ng/L based on mean measured concentrations.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP as follows:

o The SEP states that natural or reconstituted seawater of 10 to 17 °/oo salinity should be used when testing euryhaline shrimp species. The natural seawater used during the toxicity study had a salinity of 30 °/oo.

o The SEP states that most shrimp are to be tested at 22°C and the actual measured temperature should not deviate more than 1°C during the test. During this study the test temperature, measured in one replicate of the solvent control and in the 11 ng/L solutions, ranged from 23 - 25°C.

o The SEP states that a test is not acceptable if more than 5% of the control organisms die during a flow-through system. During the toxicity study, 10 percent of the solvent control organisms died.

The toxicity report did not provide the following information required by the SEP:

o The SEP recommends a 16-hour light and an 8-hour dark photoperiod with a 15- to 30-minute transition period between light and dark. The report did not state whether a 15- to 30-minute transition period between light and dark was maintained.

o The active ingredient of the test substance was not reported. However, the information provided by the EEB indicated that the active ingredient was 99%.

B. **Statistical Analysis:** The reviewer used the Toxanal computer program to calculate the LC50 values. These calculations are attached. The probit method provides a 96-hour LC50 value of 22 ng/L with a 95 percent confidence interval of 19 to 28 ng/L which is similar to that reported by the author. The slope of the toxicity curve was estimated to be 5.2.

C. **Discussion/Results:** The study results appear to be scientifically valid, however, solvent control mortality exceeded the 5 percent limit. The 96-hour LC50 value

based upon mean measured concentrations was estimated to be 22 ng/L. Therefore, ^{3}H-Avermectin B_1 is classified as very highly toxic to the mysid, Mysidopsis bahia.

D. Adequacy of the Study:

(1) **Classification:** Core

(2) **Rationale:** Solvent control mortality which exceeded the 5 percent limit, is not a severe enough deficiency to invalidate the study.

(3) **Repairability:** N/A

15. COMPLETION OF ONE-LINER: Yes, 2/8/89.

Original

Accession No. 408563-04

DATA EVALUATION RECORD

1. **CHEMICAL:** ^{3}H-Avermectin B_1
Shaughnessey No. 122804

2. **TEST MATERIAL:** ^{3}H-Avermectin B_1; Lot #L-676,863-164L010; ^{3}H-avermectin B_1 was a 7.95:1 mixture of avermectin B_{1a} and avermectin B_{1b}, with the tritium label (at the 5-position) present only in the avermectin B_{1a} fraction.

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5. **REVIEWED BY:**

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Signature: Kimberly D. Rhodes
Date: 2/8/89

6. **APPROVED BY:**

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
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Signature: P. Kosalwat
Date: 2/23/89

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature:
Date:

7. **CONCLUSIONS:** This study appears scientifically sound but does not fulfill the Guideline requirements for a 96-hour flow-through acute toxicity study for estuarine and marine shrimp. The 96-hour LC50 based upon mean measured concentrations of ^{3}H-Avermectin B_1 to the mysid (Mysidopsis bahia) was 22 ng/L. Therefore, ^{3}H-Avermectin B_1 is classified as very highly toxic to the mysid.

8. **RECOMMENDATIONS:** N/A

original

based upon mean measured concentrations was estimated to be 22 ng/L. Therefore, ^{3}H-Avermectin B_1 is classified as very highly toxic to the mysid, Mysidopsis bahia.

D. Adequacy of the Study:

(1) **Classification:** Invalid

(2) **Rationale:** Solvent control mortality exceeded the 5 percent limit.

(3) **Repairability:** N/A

15. COMPLETION OF ONE-LINER: Yes, 2/8/89.

Table 3. Concentrations tested and corresponding mortalities of mysid shrimp (Mysidopsis bahia) during the 96-hour flow-through exposure to ^{3}H-avermectin B_1.

Mean Measured Concentration (µg/L)	Cumulative Mortality (%)											
	24-hour			48-hour			72-hour			96-hour		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
Control	0	0	0	0	0	0	0	0	0	0	0	0
Solvent Control	0	10	5	0	10	5	0	10	5	10	10	10
4.2	0	0	0	0	10	5	0	10	5	0	10	5
7.7	0	0	0	0	10	5	0	10	5	0	10	5
10	0	0	0	0	0	0	10	10	10	10	20	15
16	0	10	5	10	10	10	10	10	10	20	10	15
29	10	20	15	40	30	35[a]	50	60	55[a]	70	90	80[b]

[a] All of the surviving mysids were darkened in body pigmentation.
[b] Several surviving mysids were lethargic and darkened in body pigmentation.

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

KIMBERLY RHODES H-AVERMECTIN B1 MYSIDOPSIS BAHIA 2/1/89

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
29	19	15	78.9474	.9605406
16	19	2	10.5263	3.643036E-02
10	19	2	10.5263	3.643036E-02
7.7	19	0	0	1.907348E-04
4.2	19	0	0	1.907348E-04

THE BINOMIAL TEST SHOWS THAT 16 AND 29 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 22.76986

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.1913671	22.76986	19.90228	26.74394

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.1609184	1	.1929606

SLOPE = 5.217452
95 PERCENT CONFIDENCE LIMITS = 3.12449 AND 7.310414

LC50 = 21.99033
95 PERCENT CONFIDENCE LIMITS = 18.51012 AND 27.91861

LC10 = 12.55504
95 PERCENT CONFIDENCE LIMITS = 8.952122 AND 15.17701

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Accession No. 408563-06

DATA EVALUATION RECORD

1. **CHEMICAL:** ^{3}H-Avermectin B_1
Shaughnessey No. 122804

2. **TEST MATERIAL:** ^{3}H-Avermectin B_1; Lot # 87-20014525-131, [^{3}H]L-676,863-164L011, 1022 uCi/mg; ^{3}H-avermectin B_1 was a 13.0:1 mixture of avermectin B_{1a} and avermectin B_{1b}, with the tritium label (at the 5-position) present only in the avermectin B_{1a} fraction; Chemical and radiochemical purities of ^{3}H-Avermectin B_1 was >99%.

3. **STUDY TYPE:** 28-Day Chronic Toxicity Test.
Species Tested: Mysid Shrimp (Mysidopsis bahia)

4. **CITATION:** Suprenant D.C. (1988) Chronic Toxicity of ^{3}H-Avermectin B_1 to Mysid Shrimp (Mysidopsis bahia). Prepared by Springborn Life Sciences, Wareham, Massachusetts. Submitted by Merck and Company, Inc., Rahway, New Jersey. Accession No. 408563-06.

5. **REVIEWED BY:**

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Hunter/ESE, Inc.

Signature: Kimberly D. Rhodes
Date: 2/8/89

6. **APPROVED BY:**

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KBN Engineering and
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Signature: P. Kosalwat
Date: 2/23/89

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: Daniel Rieder 4-14-89
Date: Henry T. Craven

7. **CONCLUSIONS:** This study appears scientifically sound but does not fulfill the Guideline requirements for a 28-day chronic toxicity test for estuarine and marine shrimp. The MATC of ^{3}H-avermectin B_1 for mysid shrimp was estimated to be $\geq$ 3.5 ng/L and $\leq$ 9.3 ng/L (Geometric Mean MATC = 5.7 ng/L).

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: Juvenile (≤ 24-hours old) mysids (Mysidopsis bahia) used in this test were cultured and acclimated at the testing facility. Prior to testing, mysids were maintained in natural filtered seawater at conditions compatible with those in the test, i.e., a salinity of approximately 30 °/oo, and a temperature of 25 degrees Celsius (°C). Mysids were fed live brine shrimp nauplii supplemented with Selco^{R} twice daily and Hatchfry Encapsulon^{R}, a high protein supplement, three times weekly. The mysid culture area received a regulated photoperiod of 16-hours light and 8-hours darkness. Commercial aquarium heaters were used to maintain the culture solution temperatures at 25 ± 1°C.

B. Test System: The test was conducted using an exposure system consisting of a modified Mount and Brungs (1967) proportional diluter, a temperature controlled water bath, and a set of 14 test aquaria. The test system was designed to provide five concentrations of test material, a dilution water (seawater) control and solvent control. The solvent control solution was maintained at 5.7 uL of acetone per liter of solution which was equal to the solvent concentration in the highest treatment level. Mysid retention chambers, constructed from glass petri dishes and nylon screen (363-um mesh size opening), were used to maintain the mysids during the initial phase (17 days) of the chronic exposure. Cylindrical glass isolation jars containing two 1.9-cm holes covered with nylon screens were used after day 17. The flow rate of exposure solutions to each test aquarium was approximately equivalent to 7 volume additions per 24 hours. Test aquaria were impartially positioned in a water bath containing circulating water heated by immersion coil heaters and regulated by a mercury column thermoregulator designed to maintain the test solution temperature at 25 ± 1°C.

C. Dosage: 28-day chronic flow-through test.

D. Design: Selection of ^{3}H-Avermectin B_1 concentrations for the 28-day chronic toxicity test with mysid shrimp was based on preliminary exposures of M. bahia to ^{3}H-Avermectin B_1. The test was initiated when 60 (≤ 24 hours old) mysid shrimp, were randomly distributed to each concentration or control (30 mysids per replicate). A control, solvent control and five nominal ^{3}H-Avermectin B_1 concentrations of 0.5, 1.0, 2.0, 4.0, and

8.0 ng/L were tested. When mysids had reached sexual maturity (day 17), they were redistributed within the test aquaria. Mature male/female pairs within each exposure aquaria were transferred from the retention chambers to ten glass isolation jars. The remaining mysids (after isolation of male/female pairs) were pooled and placed in a clean retention chamber within each aquarium where they were maintained for the duration of the chronic test at appropriate test concentrations. Male mysids from this pool were used to replace dead males from the paired (male/female) isolation jars. Females which died in the isolation jars were not replaced. During the first 16 days of the test, the number of dead organisms and any unusual appearance or behavior were recorded daily. After males and females had been paired on day 17, the number of dead males and females, the number of offspring produced by each individual female, and the appearance and abnormal behavior, if observed, of the adult mysids were recorded daily. At test termination, all mysids were separated into male and female groups for each replicate exposure system and were transferred into aluminum pans and dried for approximately 24 hours at 60^{o}C and then cooled in a desiccator. Individual body weights were recorded separately for each replicate of each concentration and the controls. Salinity and temperature were measured daily in the dilution water control. Dissolved oxygen concentration and pH were measured daily in each replicate of each treatment level and the controls throughout the 28-day exposure. Solution temperature was continuously monitored throughout the study in one replicate of the solvent control. Analytical determination of ^{3}H-Avermectin B_1 was performed on all treatment levels at test initiation and once weekly thereafter.

E. <u>Statistics</u>: All control and solvent data for each of the measured endpoints were compared for significant difference by analysis of variance (ANOVA). There were no differences between survival and growth of the two control groups, however a significant difference was detected between the reproductive success of the two control groups. The Chi-Square Goodness of Fit Test (Horning and Weber, 1985) was conducted and compared the observed sample distribution with a normal distribution. As a check on the assumption of homogeneity of variance implicit in parametric statistics, data for each endpoint were analyzed using Bartlett's Test (Horning and Weber, 1985). The performance at each dose level of ^{3}H-avermectin B_1 was compared with the performance of the solvent control using the William's Test (Williams, 1971, 1972), the Dunnett's Test (Dunnett, 1955, 1964),

or the Kruskal-Wallis Test (Zar, 1985; Sokal and Rohlf, 1981).

The Maximum Allowable Toxicant Concentration (MATC) was calculated by taking the geometric mean of the limits set by the lowest test concentration that showed a statistically significant effect (Lowest Observed Effect Concentration, LOEC) and the highest test concentration that showed no statistically significant difference from the control (No Observed Effect Concentration, NOEC).

12. REPORTED RESULTS: "A summary of the percent survival of adult mysids at the termination of the life cycle test is presented in Table 3 (attached). At test termination mysid survival at the highest mean measured test concentration was 10%, which was significantly less than the survival of the solvent control organisms (90%). Survival among mysids exposed to the remaining lower treatment levels (3.5 to 0.35 ng/L) ranged from 75 to 90% which was statistically comparable to the survival of the solvent control organisms." No concentration-related effects on organism survival were observed in the remaining four mean measured concentrations (3.5, 1.4, 0.76, and 0.35 ng ^{3}H-avermectin B_1/L).

"Comparison of the organism growth data (i.e. female and male dry weight), determined at test termination, established that the growth of mysids was not adversely affected at test concentrations lower than the level (9.3 ng/L) which adversely affected organism survival. Since the percentage survival of mysids exposed to the highest concentration of ^{3}H-avermectin B_1/L tested (9.3 ng/L) was significantly affected, the growth data for this concentration was not statistically analyzed."

"Mysid reproduction expressed as cumulative number of offspring per female organism per reproductive day ranged from 0.30 to 0.63 at all treatment levels and was statistically comparable to the number of offspring released by the solvent control mysids (0.55)."

"It was established that the adverse effect on survival was the most sensitive indicator of toxicity of ^{3}H-avermectin B_1 for mysid shrimp. Based on these data, the MATC of ^{3}H-avermectin B_1 for mysid shrimp was estimated to be $\leq$ 9.3 ng/L and $\geq$ 3.5 ng/L (Geometric Mean MATC = 5.7 ng/L)."

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The MATC of ^{3}H-avermectin B_1 for mysid shrimp was estimated to be $\leq$ 9.3 ng/L and $\geq$ 3.5 ng/L (Geometric Mean MATC = 5.7 ng/L).

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the ASTM procedure as follows:

o ASTM states that during the test each measured salinity should be between 15 and 30 °/oo. During this study, the salinity ranged from 30 - 32 °/oo.

o ASTM states that chronic toxicity tests with Mysidopsis bahia should be conducted at 27°C. During this study, the test temperature ranged from 24 - 26°C.

The toxicity report did not provide the following information required by the ASTM:

o Measurement of total body length was not performed.

B. Statistical Analysis: The solvent control data were used to compare the treatment levels. Significant differences in the percentage survival were determined after angular (arcsine square-root percentage) transformation of the data. Differences were determined by ANOVA. The reviewer confirmed statistical significance in reduced mysid survival at a mean measured test concentration of 9.3 ng/L.

C. Discussion/Results: The study results appear to be scientifically valid, however, statistical analysis could not be conducted on weight and reproductive success since the raw data were not susbmitted. Based on survival, the MATC of ^{3}H-avermectin B_1 for mysid shrimp was estimated to be $\leq$ 9.3 ng/L and $\geq$ 3.5 ng/L (Geometric Mean MATC = 5.7 ng/L).

D. Adequacy of the Study:

(1) Classification: Supplemental

(2) Rationale: Statistical analyses on weight and reproductive success could not be performed due to the lack of raw data.

(3) Repairability: Yes, submitt raw data on weight and reproductive success.

15. COMPLETION OF ONE-LINER: Yes, 2/8/89.

Table 3. Summary of survival and reproductive success (offspring/female/reproductive day) for the 28-day life cycle test conducted with mysid shrimp (Mysidopsis bahia) exposed to ^{3}H-avermectin B_1.

Mean Measured Test Concentration (ng/L)		Survival (%) Day 28	Reproductive Success	(N)
Control	A	93	0.81 ± 0.19	10
	B	80	0.76 ± 0.12	10
	Mean	87	0.79 ± 0.16[a]	20
Solvent Control	A	100	0.59 ± 0.16	10
	B	80	0.51 ± 0.29	10
	Mean	90	0.55 ± 0.23[a]	20
0.35	A	90	0.65 ± 0.40	10
	B	80	0.61 ± 0.26	10
	Mean	85	0.63 ± 0.33	20
0.76	A	90	0.61 ± 0.32	10
	B	90	0.24 ± 0.17	10
	Mean	90	0.42 ± 0.32	20
1.4	A	90	0.44 ± 0.20	10
	B	67	0.57 ± 0.42	10
	Mean	79	0.50 ± 0.33	20
3.5	A	67	0.25 ± 0.47	10
	B	83	0.35 ± 0.26	10
	Mean	75	0.30 ± 0.37	20
9.3	A	3	0.52 ± 0.95	10
	B	17	0.42 ± 0.50	10
	Mean	10[b]	0.47 ± 0.74	20[c]

[a] Control and solvent control data were significantly ($P \leq 0.05$) different from one another.
[b] Indicates a significant difference ($P \leq 0.05$) from the solvent control data.
[c] Due to significantly reduced survival, reproduction data for this concentration were not statistically compared to the solvent control data.

Accession #408563-06

Chronic Toxicity of ^{3}H-Avermectin B, to Mysid Shrimp (Mysidopsis bahia).

Arcsin $\sqrt{\text{Percentage}}$ Transformation of Survival

Solvent Control	A	100	→	90.0
	B	80	→	63.44
	Mean	90		76.72
0.35 ng/L	A	90	→	71.56
	B	80	→	63.44
	Mean	85		67.50
0.76 ng/L	A	90	→	71.56
	B	90	→	71.56
	Mean	90		71.56
1.4 ng/L	A	90	→	71.56
	B	67	→	54.94
	Mean	79		63.25
3.5 ng/L	A	67	→	54.94
	B	83	→	65.65
	Mean	75		60.295
9.3 ng/L	A	3	→	9.98
	B	17	→	24.35
	Mean	10		17.165

Accession #408563-06 M. bahia survival

Summary Statistics and ANOVA

Transformation: ~~None~~ Arcsin √Percentage

Group	n	Mean	s.d.	cv%
1 = control	2	76.7200	18.7808	24.5
2	2	67.5000	5.7417	8.5
3	2	71.5600	.0000	.0
4	2	63.2500	11.7521	18.6
5	2	60.2950	7.5731	12.6
6*	2	17.1650	10.1611	59.2

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minumum detectable difference for Dunnett's test = -30.224892
This difference corresponds to -39.40 percent of control

Between groups sum of squares = 4625.750800 with 5 degrees of freedom.

Error mean square = 114.066117 with 6 degrees of freedom.

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**************************************************
*                                                *
* Warning - the test for equality of variances   *
* could not be computed as 1 or more of the      *
* variances is zero.                             *
*                                                *
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Accession No. 408563-05

DATA EVALUATION RECORD

1. **CHEMICAL:** ^{3}H-Avermectin B_1
Shaughnessey No. 122804

2. **TEST MATERIAL:** ^{3}H-Avermectin B_1; Lot #L-676,863-164L012; ^{3}H-avermectin B_1 was an 11.8:1 mixture of avermectin B_{1a} and avermectin B_{1b}, with the tritium label (at the 5-position) present only in the avermectin B_{1a} fraction.

3. **STUDY TYPE:** Estuarine Organism 96-hour Flow-Through Toxicity Test. Species Tested: Mysid Shrimp (Mysidopsis bahia)

4. **CITATION:** Suprenant D.C. (1988) Acute Toxicity of ^{3}H-Avermectin B_1 to Mysid Shrimp (Mysidopsis bahia) of Different Ages Under Flow-Through Conditions. Prepared by Springborn Life Sciences, Wareham, Massachusetts. Submitted by Merck and Company, Inc., Rahway, New Jersey. Accession No. 408563-05.

5. **REVIEWED BY:**

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Supervisor, EEB/HED
USEPA

Signature: Daniel Rieder 4/14/89
Date: Henry T. Craven

7. **CONCLUSIONS:** This study appears scientifically sound, and fulfills the Guideline requirements for a 96-hour flow-through acute toxicity study for estuarine and marine shrimp. The 96-hour LC50 values, based upon mean measured concentrations of ^{3}H-Avermectin B_1, of each age group of Mysidopsis bahia ($\leq$ 1, 4, 10 and 21 days old) exposed were 20, 24, 32, and 33 ng/L, respectively. Therefore, ^{3}H-Avermectin B_1 is classified as very highly toxic to the mysid. The NOEC value for all age groups tested, was 1.3 ng/L.

8. **RECOMMENDATIONS:** N/A

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: Each specific age group of mysids (i.e., ≤ 1-, 4-, 10-, and 21-day-old) used in this toxicity test were cultured and acclimated at the testing facility. Prior to testing, mysids were maintained in natural filtered seawater under recirculating conditions. Mysids were fed brine shrimp nauplii two times daily and Hatchfry Encapsulon^{R} three times weekly. The mysid culture area received a regulated photoperiod of 16-hour light and 8-hour darkness. Commercial aquarium heaters were used to maintain the culture solution temperatures at 25 ± 1°C.

B. Test System: The test was conducted using an exposure system consisting of a modified continuous-flow (Benoit, 1982) proportional diluter, a temperature controlled water bath, and a set of 16 test aquaria. The test system was designed to provide six concentrations of test material, a dilution water (seawater) control and solvent control. The solvent control solution contained the maximum amount of acetone present in any test concentration (19 uL/L). All treatment levels and the controls were maintained in duplicate. Each glass test aquarium measured 39 x 20 x 25 centimeters (cm) with a self-starting siphon attached to a system drain. Four mysid retention chambers, constructed from glass petri dishes and nylon screen (363-um mesh size opening), were positioned in each aquarium. This system allowed the aquarium volume to fluctuate between 3.1 and 7.0 L. The flow rate of exposure solutions to each test aquaria was equivalent to 11 volume additions per 24 hours. Test aquaria were impartially positioned in a water bath containing circulating water heated by immersion coil heaters and regulated by a mercury column thermoregulator designed to maintain the test solution temperature at 25 ± 1°C.

C. Dosage: 96-hour acute flow-through test.

D. Design: Selection of ^{3}H-Avermectin B_1 concentrations for the 96-hour acute toxicity test with mysid shrimp was based on preliminary exposures of M. bahia to ^{3}H-Avermectin B_1. The test was initiated when 20 (≤ 1-, 4-, 10-, and 21-day old) mysid shrimp, were randomly distributed to each concentration or control (10 mysids per replicate). A control, solvent control and six nominal ^{3}H-Avermectin B_1 concentrations of 2.6, 6.4, 16,

40, 100, and 250 ng/L were tested. All concentrations were observed once every 24 hours for mortality and abnormal effects. The water quality parameters (dissolved oxygen, pH, salinity, and temperature) were measured and recorded daily for each replicate of the control solutions and each treatment level. Test solution temperature was continuously monitored in one replicate of the solvent control solution throughout the study. Analytical determination of ^{3}H-Avermectin B_1 was performed on all treatment levels at 0 and 96 hours using radiometric analysis.

E. Statistics: The mean measured concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate the median lethal concentrations (LC50) and 95% confidence intervals for each 24-hour interval of the exposure period. LC50 values were empirically estimated as being greater than the highest concentration tested when no test concentrations caused 50% or more mortalities. If at least one test concentration caused mortality of greater than or equal to 50 % of the test population, then a computer program (Stephan, 1977, 1982) was used to calculate the LC50 values and 95% confidence intervals.

12. REPORTED RESULTS: "The mean measured test concentrations, the corresponding mortalities and the observations made during the 96-hour test are presented in Table 3 (attached)." Analytical determination of test concentrations resulted in mean measured concentrations of 1.3, 4.3, 11, 21, 52, and 98 ng/L. The mean measured concentrations were 39 to 69% of the nominal concentrations. "After 96 hours, 100% mortality was observed among all age groups (i.e., $\leq$ 1-, 4-, 10-, and 21-days old) of mysids exposed to the highest mean measured concentration (98 ng/L) of ^{3}H-avermectin B_1 tested. Some mortality was recorded at the next three treatment levels for most age groups and no mortality (all age groups) was observed in the lowest test concentration, 1.3 ng/L. The control mortality for all test organisms was $\leq$ 5% for the duration of the 96-hour test." The 96-hour LC50 values, based on mean measured concentrations, for each age group of mysids ($\leq$ 1-, 4-, 10-, and 21-day-old) exposed are 20, 23, 26, and 26 ng/L, respectively. "The 96-hour LC50 values established that the test organisms of various age groups are similar in sensitivity after 96 hours of exposure to ^{3}H-avermectin B_1. At the earlier time intervals (i.e., 48 and 72 hours), however, the LC50 values suggest that the younger organisms may be more sensitive to the test material. The No Observed Effect Concentration (NOEC) for all age groups of mysid shrimp exposed to ^{3}H-avermectin B_1 was 1.3 ng/L. Based on criteria established by U.S. EPA (1985), ^{3}H-avermectin B_1

would be classified as very highly toxic to each of the age groups of mysid shrimp tested."

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 96-hour LC50 values, based on mean measured concentrations, for each age group of mysids (≤ 1-, 4-, 10-, and 21-day-old) exposed were estimated to be 20, 23, 26, and 26 ng/L, respectively. The No Observed Effect Concentration (NOEC) through 96 hours was 1.3 ng/L for each age group of mysids exposed.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP as follows:

o The SEP states that natural or reconstituted seawater of 10 to 17 °/oo salinity should be used when testing euryhaline shrimp species. The natural seawater used during the toxicity study had a salinity of 30 - 31 °/oo.

o The SEP states that most shrimp are to be tested at 22°C and the actual measured temperature should not deviate more than 1°C during the test. During this study, the test temperature ranged from 24 - 26°C.

o Test concentrations were prepared in a 40 percent dilution series; the SEP states that each concentration should be at least 60 percent of the next highest concentration.

The toxicity report did not provide the following information required by the SEP:

o The active ingredient of the test substance was not reported. However, the information obtained from the EEB indicated that the active ingredient was 99%.

o The SEP recommends a 16-hour light and an 8-hour dark photoperiod with a 15- to 30-minute transition period between light and dark. The report did not state whether a 15- to 30-minute transition period between light and dark was maintained.

B. Statistical Analysis: The reviewer used the Toxanal computer program to calculate the LC50 values. These calculations are attached. The 96-hour LC50 value for mysids ≤ 24-hours old was calculated by the moving average method to be 20 ng/L with a 95 percent confidence interval of 15 to 27 ng/L which is the same as reported by the author. The 96-hour LC50 value for 4-day old mysids was calculated by the moving average method to be 24 ng/L with a 95 percent confidence interval of 17 to 33 ng/L which is similar to that reported by the author. The 96-hour LC50 value for 10-day old mysids was calculated by the moving average method to be 32 ng/L with a 95 percent confidence interval of 23 to 49 ng/L which is higher than that reported by the author. The 96-hour LC50 value for 21-day old mysids was calculated by the moving average method to be 33 ng/L with a 95 percent confidence interval of 24 to 48 ng/L which is higher than that reported by the author.

C. Discussion/Results: The study results appear to be scientifically valid. The 96-hour LC50 values, based upon mean measured concentrations, for each age group of mysids (≤ 1-, 4-, 10-, and 21-days old) exposed were estimated to be 20, 24, 32, and 33 ng/L, respectively. The No Observed Effect Concentration (NOEC) through 96 hours was 1.3 ng/L for each age group of mysids exposed. Therefore, ^{3}H-Avermectin B_1 is classified as very highly toxic to each of the age groups of the mysid, Mysidopsis bahia tested.

D. Adequacy of the Study:

(1) Classification: Core

(2) Rationale: N/A

(3) Repairability: N/A

15. COMPLETION OF ONE-LINER: Yes, 2/8/89.

Table 3. Concentrations tested and corresponding mortalities of mysid shrimp (Mysidopsis bahia) during the 96-hour flow-through exposure to ^{3}H-avermectin B_1.

Mean Measured Concentration (ng/L)	≤ 1 Day Old Cumulative Mortality (%)											
	24-hour			48-hour			72-hour			96-hour		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
Control	0	0	0	0	0	0	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0	0	0	0	0	0	0
1.3	0	0	0	0	0	0	0	0	0	0	0	0
4.3	0	0	0	0	0	0	0	0	0	10	20	15
11	0	0	0	0	0	0	0	0	0	30	10	20[c]
21	0	0	0[a]	0	10	5	0	20	10	10	30	20
52	0	0	0[b]	30	40	35[c]	80	60	70[a]	90	90	90[c]
98	50	0	25[b,c]	100	80	90[c]	100	100	100	100	100	100

	4 Day Old Cumulative Mortality (%)											
	24-hour			48-hour			72-hour			96-hour		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
Control	0	0	0	0	0	0	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0	0	10	5	0	10	5
1.3	0	0	0	0	0	0	0	0	0	0	0	0
4.3	0	0	0	0	0	0	0	0	0	30	10	20
11	0	0	0	0	0	0	0	10	5	20	20	20
21	0	0	0	0	0	0	0	10	5	10	30	20
52	0	0	0	30	30	30	50	50	50	60	70	65[a,d]
98	0	0	0	100	70	85[a]	100	100	100	100	100	100

Table 3. Continued.

Mean Measured Concentration (ng/L)	10 Day Old Cumulative Mortality (%)											
	24-hour			48-hour			72-hour			96-hour		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
Control	0	0	0	0	0	0	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0	0	0	0	0	0	0
1.3	0	0	0	0	0	0	0	0	0	0	0	0
4.3	10	10	10	10	10	10	10	10	10	20	20	20
11	0	0	0	0	0	0	0	10	5	10	10	10
21	0	0	0	10	10	10	10	10	10	20	10	15
52	0	0	0	10	10	10	40	60	50	50	80	65
98	0	0	0[c]	100	50	75[c]	100	100	100	100	100	100

	21 Day Old Cumulative Mortality (%)											
	24-hour			48-hour			72-hour			96-hour		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
Control	0	0	0	0	0	0	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0	0	0	0	0	0	0
1.3	0	0	0	0	0	0	0	0	0	0	0	0
4.3	10	0	5	10	10	10	10	10	10	20	10	15
11	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	10	0	5[d]	10	0	5[d]	40	10	25[b]
52	0	0	0[a]	20	20	20	50	60	55[b]	70	80	75[b]
98	10	0	5	50	50	50	80	80	80[c]	100	100	100

[a] One of the surviving mysids was lethargic.
[b] Several of the surviving mysids were darkened in body pigmentation.
[c] Several of the surviving mysids were lethargic.
[d] One mysid was darkened in body pigmentation.

^{3}H-Avermectin B.
96-Hour LC-50 $\leq$ 1 Day old mysids

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KIMBERLY RHODES 3H-AVERMECTIN B1 MYSIDOPSIS BAHIA 02-06-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
98	20	20	100	9.536742E-05
52	20	18	90	2.012253E-02
21	20	4	20	.5908966
11	20	4	20	.5908966
4.3	20	3	15	.1288414
1.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 21 AND 52 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 30.51335

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	4.506168E-02	19.92877	14.93263	27.32767

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.6763403	3.531909	6.898701E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.416815
95 PERCENT CONFIDENCE LIMITS = .4292286 AND 4.404403

LC50 = 22.01686
95 PERCENT CONFIDENCE LIMITS = 5.6996 AND 84.426

LC10 = 6.565539
95 PERCENT CONFIDENCE LIMITS = 1.811056E-02 AND 15.0399

^{3}H. Avermectin B$_1$

96-Hour LC50 4-day old mysids

NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE
OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY.
THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

KIMBERLY RHODES 3H-AVERMECTIN B1 MYSIDOPSIS BAHIA 02-06-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
98	20	20	100	9.536742E-05
52	20	13	65	13.1588
21	20	4	20	.5908966
11	20	4	20	.5908966
4.3	20	4	20	.5908966
1.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 21 AND 98 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 38.81503

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	4.714533E-02	23.51604	17.471	33.10359

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.690222	3.327818	9.850562E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.864056
95 PERCENT CONFIDENCE LIMITS = .3064568 AND 3.421655

LC50 = 26.62147
95 PERCENT CONFIDENCE LIMITS = 7.045132 AND 196.597

LC10 = 5.545259
95 PERCENT CONFIDENCE LIMITS = 2.901212E-03 AND 14.5741

3H-Avermectin B1
96-Hour LC50 10-day old mysids

KIMBERLY RHODES 3H-AVERMECTIN B1 MYSIDOPSIS BAHIA 02-06-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
98	20	20	100	9.536742E-05
52	20	13	65	13.1588
21	20	3	15	.1288414
11	20	2	10	2.012253E-02
4.3	20	4	20	.5908966
1.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 21 AND 98 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 40.19604

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	9.753808E-02	32.41923	23.46331	48.5746

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	1.047357	4.862193	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.971362
95 PERCENT CONFIDENCE LIMITS =-4.613889E-02 AND 3.988864

LC50 = 29.80213
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 6.761184
95 PERCENT CONFIDENCE LIMITS = 0 AND 19.6772

3H-Avermectin B1

96-Hour LC50 21-day old mysids

KIMBERLY RHODES 3H-AVERMECTIN B1 MYSIDOPSIS BAHIA 02-06-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
98	20	20	100	9.536742E-05
52	20	15	75	2.069473
21	20	5	25	2.069473
11	20	0	0	9.536742E-05
4.3	20	3	15	.1288414
1.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 21 AND 52 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 33.04542

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.0864313	32.939	24.27973	48.11614

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	1.081974	5.330023	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.439478
95 PERCENT CONFIDENCE LIMITS =-9.801793E-02 AND 4.976974

LC50 = 28.07993
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 8.468232
95 PERCENT CONFIDENCE LIMITS = 0 AND 22.08503