Appendix I. Summary of Ecotoxicity Data

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1. Introduction

This appendix is a summary of the data being evaluated for ecological effects on aquatic organisms. Each section consists of several sets of data. Tables presenting the data submitted with the application for registration are presented first. The lowest value from this table is used to set a "benchmark" that is used in evaluating the data from the open literature—from EPA's ECOTOX database. Only the studies associated with the ECOTOX data that have values less than these benchmarks are examined further. If any of these latter values are acceptable, then that value becomes the final value used in the risk assessment for that particular taxonomic group.

In general, studies from the open literature do not provide sufficient documentation for evaluating all or most of the OPPTS Guideline evaluation criteria. Consistent with the Overview Document (USEPA 2004), if sufficient documentation was provided to judge that the open literature studies were scientifically valid and acceptable endpoints were determined that were below the values available in submitted studies, they were typically classified as supplemental-but acceptable for quantitative use. If sufficient documentation was not provided to comprehensively judge the scientific validity of the study but no information existed that suggested the study would be unacceptable, these studies were typically classified as supplemental-acceptable for qualitative use.

2. Toxicity to Aquatic Animals

2.1. Freshwater Fish, Acute

Submited Data for Freshwater Fish Acute Values
Two freshwater fish toxicity studies using the TGAI ingredient are required to establish the toxicity of a pesticide to freshwater fish. One study should use a coldwater species (preferably the rainbow trout), and the other should use a warmwater species (preferably the bluegill sunfish). Data are needed for the typical end-use product (TEP) if applied directly to water, the maximum EEC≥0.5 LC_{50}, or the end product enhances toxicity. Thiobencarb is applied directly to water when used on rice. Results of these tests are given in Table I 1.

Table I 1. Summary of submitted acute toxicity studies on freshwater fish

Taxon **TGAI/TEP** **Method**	**Duration and Endpoint (95% C.I.)**	**MRID or Source and Study Classification DER Date**	**Comments**
Bluegill Sunfish			
Bluegill sunfish *Lepomis macrochirus* TGAI 94% Static/ nominal	96-hr LC_{50} = 2600 µg ai/L	MRID 00080859 Supplemental-qualitative 08/08/1980	Incomplete reporting of test procedures. No raw data included in study report.
Bluegill *Lepomis macrochirus* TEP 95.5% Static/ nominal	96-hr LC_{50} = 2480 µg ai/L	MRID 00080851 Supplemental-qualitative 07/23/1980	Formulation IMC-3590 Supplemental because of inadequate reporting of results.
Bluegill *Lepomis macrochirus* TEP 10% Static/ measured	96-hr LC_{50} = 560 (330-1200) µg ai/L	MRID 00050665 Acceptable 12/02/1980	Formulation Bolero 10G (granular) Treatments: <0.05, 0.33, 0.65, 1.2, 2.2, 3.5 mg/L mean measured 33-39% of nominal Fish were smaller than recommended.
Bluegill Sunfish *Lepomis macrochirus* TEP 84.0% Static/ nominal	96-hr LC_{50} = 1660 µg ai/L	MRID 00080851 Supplemental-qualitative 07/23/1980	Formulation Bolero 8EC Supplemental because of inadequate reporting of results.
Bluegill sunfish *Lepomis*	96-hr LC_{50} = 1700 (1200 - 2300) µg ai/L	MRID 139051 Supplemental	Formulation Bolero 8EC Review of open literature study.

Taxon TGAI/TEP Method	Duration and Endpoint (95% C.I.)	MRID or Source and Study Classification DER Date	Comments
macrochirus TEP 85.2%		03/26/1984	
Rainbow Trout			
Rainbow trout *Oncorhynchus mykiss* TEP 85.2%	96-hr LC_{50} = 1200 (700 - 1600) µg ai/L	MRID 139051 Supplemental 03/26/1984	Formulation Bolero 8EC Review of open literature study.
Rainbow Trout *Oncorhynchus mykiss* TEP 84.0% Static/ nominal	96-hr LC_{50} = 1050 µg ai/L	MRID 00080851 Supplemental-qualitative 07/23/1980	Formulation Bolero 8EC Supplemental because of inadequate reporting of results.
Rainbow Trout *Oncorhynchus mykiss* TEP 95.5% Static/ nominal	96-hr LC_{50} = 1150 µg ai/L	MRID 00080851 Supplemental-qualitative 07/23/1980	Formulation IMC-3590 Supplemental because of inadequate reporting of results.
Rainbow Trout *Oncorhynchus mykiss* TEP 10% Static/ measured	96-hr LC_{50} = 1500 (1200- 1900) µg ai/L	MRID 00050664 Acceptable 12/02/1980	Formulation Bolero G (granular) Treatments: <0.05, 0.19, 0.46, 1.2, 1.9, 3.4 mg/L mean measured, 19-34% of nominal
Channel Catfish			
Channel Catfish *Ictalurus punctatus* TEP 84.0% Static/ nominal	96-hr LC_{50} = 2290 µg ai/L	MRID 00080851 Supplemental-qualitative 07/23/1980	Formulation Bolero 8EC Supplemental because of inadequate reporting of results.
Channel Catfish *Ictalurus punctatus* TEP 95.5% Static/ nominal	96-hr LC_{50} = 2280 µg ai/L	MRID 00080851 Supplemental-qualitative 07/23/1980	Formulation IMC-3590 Supplemental because of inadequate reporting of results.
Channel Catfish *Ictalurus punctatus* TEP 85.2%	96-hr LC_{50} = 2300 (1200 - 4400) µg ai/L	MRID 139051 Supplemental 03/26/1984	Formulation Bolero 8EC Review of open literature study.
Other Species			
Carp *Cyprinus carpio* TGAI 94% Static/ nominal	96-hr LC_{50} = 2800 µg ai/L	MRID 00080859 Supplemental-qualitative 08/08/1980	Incomplete reporting of test procedures.
White sturgeon *Acipenser*	96-hr LC_{50} = 260 (230-300) µg/L	(Bailey, 1984) MRID	28-day old fish. The study did not report the percent active ingredient of the

transmontanus TGAI NR Flow-through/ measured		40651315 Qualitative	material tested. Not enough information was available describing the preparation of the stock solutions. Fish were fed during the study.

Abbreviations: NR = not reported; TGAI = technical grade active ingredient; TEP = typical end-use product; MRID = Master Record Identification

The only studies conducted using the TGAI (or TEP with greater than 95% active ingredient (ai)) were considered supplemental or qualitative and had incomplete reporting of the test procedures. The supplemental results of these studies indicated that the 96-hr LC_{50} values for carp, bluegill sunfish, channel catfish, and rainbow trout ranged from 1180 - 2800 µg ai/L (Table I 14). The only fully acceptable submitted studies on the acute toxicity of thiobencarb to fish were conducted with Bolero 10 G. The 96-hour LC_{50} for bluegill sunfish exposed to Bolero 10 G (10% ai) was 560 µg ai/L. This result is inconsistent with the results of other acute tests for the bluegill sunfish where LC_{50} values for TEPs ranged from 1660 – 2800 µg ai/L. Results of tests with the technical end-use product (TEP) or TGAI with less than 91% ai ranged from 260 – 2480 µg ai/L indicating that thiobencarb formulations are highly toxic to moderately toxic to freshwater fish. The most sensitive species was the white sturgeon and this value may only be used qualitatively due to incomplete information available in the test report. As little information is available on the studies conducted on the TGAI, it is not possible to determine whether the TEP or TGAI is more toxic. As the TGAI studies are not as reliable as the TEP studies, results from the TEP will be used quantitatively in the risk assessment and it will be assumed that the toxicity to the formulation and TGAI are similar. This assumption is supported from open literature studies (Harrington, 1990).

Thiobencarb and thiobencarb formulations are moderately to highly toxic to freshwater fish. The freshwater fish acute toxicity testing requirement using technical end product (Guideline 72-1) is fulfilled (MRID 50665, 50664).

Additional Open Literature Information (ECOTOX) for Freshwater Fish Acute Values

All of the open literature acute values from EPA's ECOTOX database for freshwater fish are presented in Table I 2. These data are primarily for an exposure duration of 4 days; however, other durations were included (*e.g.*, 1 or 2 day exposures) if there was no 4 day LC_{50} for a particular test. Table I 2 contains the acute LC_{50} values that are less than the benchmark of 560 µg ai/L. When LC_{50} values for multiple exposure durations from the same test were available, only the value for 4 day exposure was included. There were 45 LC_{50} values from 13 open literature studies representing 17 freshwater fish species. Five of these values were below the freshwater fish benchmark (560 µg/L) that was based on the information provided by the registrant. The lowest of these more sensitive acute LC_{50} values is 260 µg a.i./L (Bailey, 1984) for the white sturgeon, *Acipenser transmontanus*[1]. This study was evaluated and considered qualitative because the percent active ingredient was not reported for the test material and not enough information was available on preparation of the test solutions. Several toxicity studies

[1] A study report describing this study was also submitted to the Agency.

were conducted examining toxicity to the striped bass (Fujimura *et al.*, 1991). The studies examining toxicity to the striped bass with endpoints lower than those submitted did not include solvent controls. These 96-hour LC_{50} values ranged from 430 – 550 µg/L. Control mortality was greater than 10% in the test with the lowest endpoint of 430 µg/L and this value may only be used qualitatively. Solvent controls were completed for the studies on striped bass completed in 1989 and no differences were observed between the negative and solvent controls. Therefore, it may be assumed that the solvents used did not influence the results and the 96-hr LC_{50} of 440 µg/L may be used quantitatively. The other endpoints reported in the study are also considered quantitative and may be used to calculate an acute-to-chronic ratio and/or risk quotient.

Table I 2. Summary of freshwater fish acute data from EPA's ECOTOX database and other open literature studies. The lowest values were evaluated until a value was found that could be used quantitatively.

Genus	Species	Common Name	Dur (days)	LC_{50} value (95% C.I.) µg/L	Exp Type	Ecotox Ref #/ Source	Comment
Acipenser	*Transmontanus*	White Sturgeon	4	260 (230-300)	F	(Bailey, 1984)	Supplemental - qualitative TGAI, measured
Morone	*saxatilis*	Striped bass	4	430	F	15472	Supplemental - qualitative Bolero 8EC (85.2% ai) measured
Morone	*saxatilis*	Striped bass	4	440 (400-500)	F	15472	Supplemental - quantitative Bolero 8EC (85.2% ai) measured
Morone	*saxatilis*	Striped bass	4	480 (440-530)	F	15472	Supplemental - quantitative Bolero 8EC (85.2% ai) measured
Morone	*saxatilis*	Striped bass	4	550	F	15472	Supplemental - quantitative Bolero 8EC (85.2% ai) measured
The values above this line are lower than submitted values – the most sensitive value was 560 µg ai/L.							
Morone	*saxatilis*	Striped bass	4	570 (500-650)	F	15472	Supplemental - quantitative Bolero 8EC (85.2% ai) measured
Poecilia	*reticulata*	Guppy	1	610	S	20421	

Genus	Species	Common Name	Dur (days)	LC_{50} value (95% C.I.) µg/L	Exp Type	Ecotox Ref #/ Source	Comment
Morone	*saxatilis*	Striped bass	4	640	F	15472	Supplemental –quantitative* measured
Morone	*saxatilis*	Striped bass	4	690	F	15472	Supplemental –quantitative* measured
Morone	*saxatilis*	Striped bass	4	720	F	15472	Supplemental - qualitative* measured
Morone	*saxatilis*	Striped bass	4	760	F	12136	Supplemental - qualitative* measured
Oncorhynchus	*tshawytscha*	Chinook salmon	4	760	F	12136	measured
Morone	*saxatilis*	Striped bass	4	760	F	15472	measured
Morone	*saxatilis*	Striped bass	4	770	F	15472	Supplemental –quantitative* measured
Oncorhynchus	*mykiss*	Rainbow trout,donaldson trout	4	790	F	12136	
Morone	*saxatilis*	Striped bass	4	830	F	15472	Supplemental - qualitative* measured
Morone	*saxatilis*	Striped bass	4	840	F	15472	Supplemental - qualitative* measured
Morone	*saxatilis*	Striped bass	4	880	F	15472	Supplemental - qualitative* measured
Anguilla	*japonica*	Japanese eel	2	890	S	5016	
Morone	*saxatilis*	Striped bass	4	910	F	15472	Supplemental - qualitative* measured
Morone	*saxatilis*	Striped bass	4	920	F	15472	Supplemental - qualitative* measured
Morone	*saxatilis*	Striped bass	4	1000	F	15472	Supplemental - qualitative* measured
Morone	*saxatilis*	Striped bass	4	1000	F	15472	Supplemental - qualitative* measured
Tilapia	*nilotica*	Nile tilapia	2	1135	S	20421	
Oncorhynchus	*mykiss*	Rainbow trout,donaldson trout	4	1200	S	15172	

Genus	Species	Common Name	Dur (days)	LC_{50} value (95% C.I.) µg/L	Exp Type	Ecotox Ref #/ Source	Comment
Cyprinus	*carpio*	common carp	4	1420	S	6577	
Ctenopharyngodon	*idella*	Grass carp, white amur	2	1510	S	5016	
Lepomis	*macrochirus*	Bluegill	4	1700	S	15172	
Ictalurus	*punctatus*	Channel catfish	4	1800	F	12136	measured
Cyprinus	*carpio*	common carp	3	1850	S	6577	
Cyprinus	*carpio*	common carp	2	1930	S	5016	
Oryzias	*latipes*	Medaka, high-eyes	4	1960	S	55229	
Oreochromis	*mossambicus*	Mozambique Tilapia	2	1990	S	5016	
Cyprinus	*carpio*	common carp	2	2350	S	6577	
Hypophthalmichthys	*nobilis*	Carp	2	2450	S	5016	
Misgurnus	*anguillicaudatus*	Oriental weatherfish	2	2540	S	5016	
Cyprinus	*carpio*	common carp	1	2950	S	6577	
Oryzias	*latipes*	Medaka, high-eyes	4	3000	S	89099	measured
Oryzias	*latipes*	Medaka, high-eyes	4	3822	S	55229	
Oryzias	*latipes*	Medaka, high-eyes	2	4100	S	17989	
Oryzias	*latipes*	Medaka, high-eyes	1	10000	S	17989	
Anguilla	*anguilla*	Common eel	4	13200	S	61820	
Anguilla	*anguilla*	Common eel	3	17000	S	61820	
Anguilla	*anguilla*	Common eel	2	21700	S	61820	
Anguilla	*anguilla*	Common eel	1	25700	S	61820	

Abbreviations: Exp = exposure; Dur = duration; S = static; F = flow-through; EC = emulsifiable concentrate; TGAI = technical grad active ingredient; TEP = typical end-use product; C.I. = Confidence interval

*The studies that were reported in this study that had both a solvent and negative control were reported as quantitative.

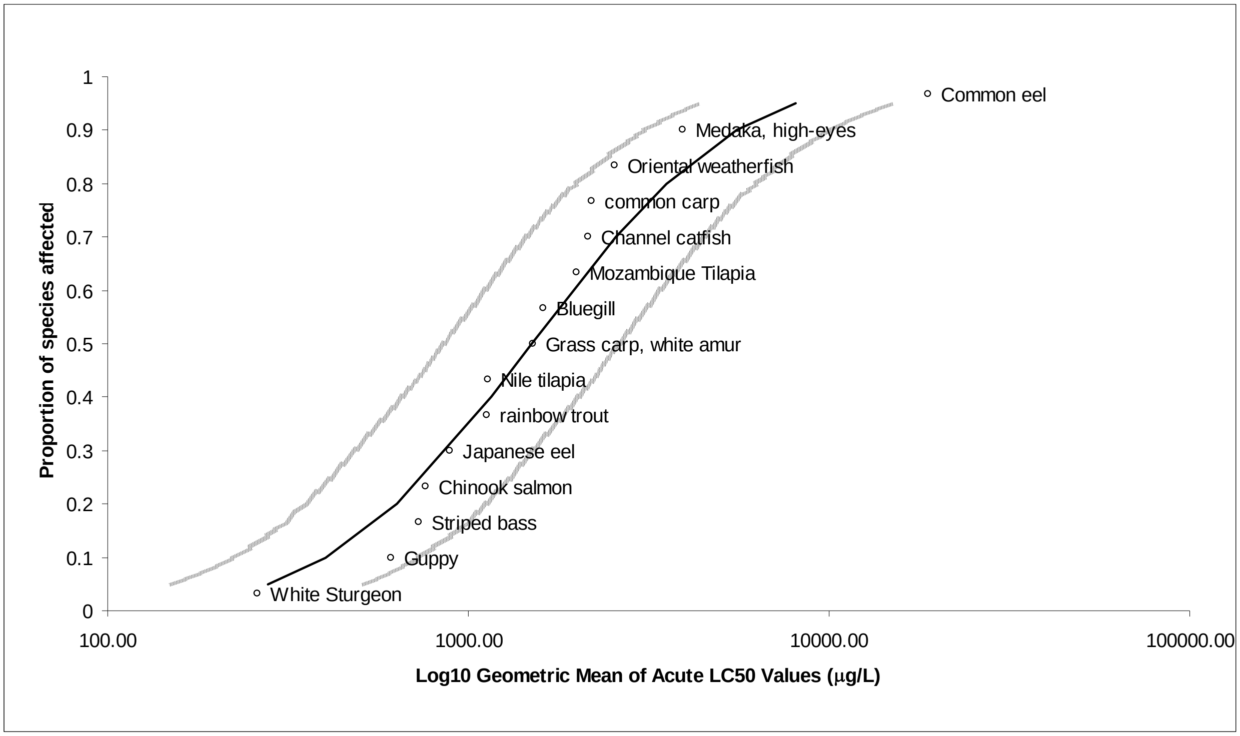

Figure I 1. Species sensitivity distribution on the acute toxicity of thiobencarb (TEP and TGAI) to freshwater fish using all data accepted from the ECOTOX database and submitted studies.[2]

2.2. Freshwater Fish, Chronic

A freshwater fish early life-stage test using the TGAI is required for thiobencarb because the end-use product may be applied directly to water and thiobencarb is expected to be transported to water from the intended use site (rice) and because the following conditions are met: (1) some aquatic acute LC_{50} and EC_{50} are less than 1 mg/l, (2) EECs in water (based on measured concentrations) were greater than 1% of acute LC_{50} and EC_{50} values, and (3) the half-life in water is greater than 4 days. A fish full life-cycle was submitted for the fathead minnow. The study should have a corresponding acute study for the same species. No acute toxicity data were submitted for the fathead minnow. Additionally, the study only had two replicates. The low number of replicates and variability in the endpoints may have resulted in an inability to statistically detect differences between treatments and controls and thus may overestimate toxicity endpoints. Observed results suggested that if more replicates were available a difference may have been statistically significant between the control and 53 µg ai/L treatment group.

[2] This species sensitivity distribution was developed using the Species Sensitivity Generator version 1.0 available from Causal Analysis/Diagnosis Decision Information System (CADDIS).

Table I 3. Summary of chronic toxicity studies for freshwater fish

Taxon TGAI/TEP Test System Study type/ nominal or measured	**Duration and Endpoint (95% C.I.)**	**MRID or Source and Study Classification DER Date**	**Comments**
Fathead minnow *Pimephales promelas* TGAI 96.5% Flow-through/ measured	Full Life Cycle (260 days) NOAEC/LOAEC = 53/ 110 µg ai/L (F_0 8-week survival, F_0 maturation (week 24) wet weight (Males), F_0 maturation (week 37) wet weight (Females and Males), F_0 eggs/female, F_0 eggs/spawn, F_1 hatching success, F_1 4-week survival, F_1 4-week length)	MRID 45695101 Supplemental	Treatments: 5.8, 13, 26, 53, and 110 µg ai/L mean measured; All CVs < 20% Only 2 replicates. The low number of replicates and variability in the endpoints may have resulted in an inability to statistically detect differences between treatments and controls. Toxicity endpoints may be overestimated. Observed results suggested that if more replicates were available a difference may have been statistically significant from control for the 53 µg ai/L treatment group.
Chinook salmon *Oncorhynchus tshawytscha* TEP 85.2 Flow-through/ measured	NOAEC/LOAEC = 28/ 49 µg ai/L (wet weight, length) 140/ 250 µg ai/L (survival)	E0015472 (Faggella and Finlayson, 1988) Quantitative	Formulation: Bolero 8EC Embryo-to-fry study Hatching success was not determined and this value may underestimate risk due to effects on hatching success.
Striped bass *Morone saxatilis* TEP 85.2% Flow-through/ measured	NOAEC/LOAEC = 21/ 36 µg ai/L (1-hour posthatch, survival) NOAEC/LOAEC <23/ 23 µg ai/L (9-day posthatch, survival) NOAECL/LOAEC =58/ 91 µg ai/L (8-day posthatch, survival)	E0015472 (Fujimura *et al.*, 1991) Quantitative	Formulation: Bolero 8EC Early life stage study. Hatching success was not determined and this value may underestimate risk due to effects on hatching success.

Additional Open Literature Information (ECOTOX) for Freshwater Fish Chronic Values

Open literature data are available for the Chinook salmon and Striped bass, with the most sensitive endpoint reported for the Striped bass (ECOTOX reference number 15472). The NOAEC and LOAEC from that study for survival was 21 and 36 µg/L, respectively. A NOAEC was not determined in an early life stage study with striped bass at 8-day post hatch at the test initiation. At the lowest measured test concentration of 23 µg/L survival was 63% as compared to 85% in controls. Values in Table I 4 were used to calculate acute-to-chronic (ACR) ratios that could be used to estimate chronic toxicity values if needed.

Table I 4. Calculation of the acute-to-chronic ratio for freshwater fish exposed to thiobencarb

Species	Highest LC_{50} value (µg ai/L)	Lowest Chronic NOAEC (µg ai/L)	Acute-to-chronic ratio	Ecotox Reference Number and Citation	Comment
Chinook salmon	760 (n=1)	28	27	E12136 (Finlayson and Faggella, 1986) E15472 (Fujimura *et al.*, 1991)	E12136 was not reviewed and not used in risk quotient calculations.*
Striped Bass	770	21	37	E15472 (Fujimura *et al.*, 1991)	Quantitative

*If the ACR was higher than the Striped Bass value it would be reviewed and possibly used to estimate chronic values.

2.3. Freshwater Invertebrates, Acute

A freshwater aquatic invertebrate toxicity test using the TGAI is required to assess the toxicity of a pesticide to freshwater invertebrates. Data are needed for TEP if applied directly to water, the maximum EEC≥0.5 LC_{50}, or the end product enhances toxicity. The preferred test organism is *Daphnia magna*, but early instar amphipods, stoneflies, mayflies, or midges may also be used. Results of this test are tabulated in Table I 5. The results indicate that thiobencarb is moderately to highly toxic to aquatic invertebrates on an acute basis, with LC_{50} values ranging from 101.2 to 6500 µg a.i./L. (see Table I 5). No open literature values resulted in more sensitive acute 96-hour LC_{50} values for freshwater invertebrates (Table I 6 and Figure I 2). Available data indicate that invertebrates have either similar to or less sensitivity to the TEP as compared to the TGAI. The 48-hr LC_{50} for the water flea is 101.2 µg ai/L (95% Confidence Interval, C.I., is 73.8 – 138.7 µg ai/L) and 210.7 µg ai/L (95% C.I. is 175.7 - 252.7 µg ai/L) for the TGAI and TEP, respectively. The endpoint for the TGAI is the lowest available value and will be used in the risk assessment.

Table I 5. Summary of submitted acute toxicity studies for freshwater invertebrates

Taxon TGAI/TEP Test System Study type/ nominal or measured	Duration and Endpoint (95% C.I.)	MRID or Source and Study Classification DER Date	Comments
Water flea			
Water flea *Daphnia magna* TGAI 94.4% Static/ measured	48-hr LC_{50} = 101.2 (73.8 – 138.7) µg ai/L Slope: NA	MRID 0025788 Acceptable 06/09/1980	Treatments: 0.072, 0.085, 0.150 mg/L
Water flea *Daphnia magna*	48-hr LC_{50} = 210.7 (175.7 - 252.7) µg ai/L	MRID 00079118	Formulation Bolero 8EC Treatments: 0.15, 0.26, 0.43 mg/L

Taxon TGAI/TEP Test System Study type/ nominal or measured	Duration and Endpoint (95% C.I.)	MRID or Source and Study Classification DER Date	Comments
TEP 82.25% Static/ measured		Supplemental 12/05/1990	
Water flea *Daphnia magna* TEP Flow-through/ measured	48-hr LC_{50} >1200 µg ai/L	MRID 00050666 Supplemental 12/02/1980	Treatments: <0.02, 0.16, 0.15, 0.24, 0.44, 0.72, 1.2 mg/L mean measured. 67-160% of nominal Clumping observed at 0.46 (0.39-0.54) mg/L mean measured concentrations. 45% mortality observed at 1.2 mg/L
Water flea *Daphnia magna* TEP 85.2%	48-hr LC_{50} = 1200 (400 – 3100) µg ai/L	MRID 139051 Supplemental 03/26/1984	Formulation Bolero 8EC Review of open literature study.
Scud			
Scud *Gammarus pseudolimimaeus* TEP 85.2%	48-hr LC_{50} = 1000 (600 -1700) µg ai/L	MRID 139051 Supplemental 03/26/1984	Formulation Bolero 8EC Review of open literature study.
Scud *Gammarus pseudolimimaeus* TEP 95.5% Static/ nominal	96-hr LC_{50} = 720 µg ai/L	MRID 00080851 Supplemental 07/23/1980	Formulation IMC-3590 Supplemental because of inadequate reporting of results.
Scud *Gammarus pseudolimimaeu* TEP 84.0% Static/ nominal	96-hr LC_{50} = 1000 µg ai/L	MRID 00080851 Supplemental 07/23/1980	Formulation Bolero 8EC Supplemental because of inadequate reporting of results.
Crayfish			
Crayfish *Orconectes naois* TEP 95.5% Flow-through/ measured	96-hr LC_{50} = 2000 µg ai/L	MRID 00080851 Supplemental 07/23/1980	Formulation IMC-3590 Supplemental because of inadequate reporting of results.
Crayfish *Orconectes naois* TEP 85.2%	48-hr LC_{50} = 2000 (1400 - 3600) µg ai/L	MRID 139051 Supplemental 03/26/1984	Formulation Bolero 8EC Review of open literature study.
Red Crayfish *Procambarus clarki* TEP 85.2%	48-hr LC_{50} = 6500 (5700 - 7100) µg ai/L	MRID 139051 Supplemental 03/26/1984	Formulation Bolero 8EC Review of open literature study.
Other Species			
Oligochaete	96-hr LC_{50} = 2540	MRID	Treatments: 1.08, 1.33, 1.68, 2.19, 2.91,

Taxon TGAI/TEP Test System Study type/ nominal or measured	Duration and Endpoint (95% C.I.)	MRID or Source and Study Classification DER Date	Comments
Lumbriculus varirgatus TGAI 97.2% Static renewal/ measured	(2390-2700) µg ai/L Slope = 9.66 (7.46-11.9) NOAEL = 1690 µg ai/L	44628601 Supplemental 06/10/2002	3.61 mg/L The study was classified as supplemental because there is not a guideline for the study.
Midge *Chironomid tentans* TGAI 97.2% Static/ measured	48-hr LC_{50} = 364 (322 - 413) µg ai/L Slope = 3.52 (2.88-4.16) NOAEC = 135 µg ai/L (mortality)	MRID 44628602 Supplemental 10/21/2002	Treatments: 0.09, 0.135, 0.195, 0.305, 0.43, 0.645, 1.00, 1.465 mg/L measured The study was classified as supplemental because the midge is not a recommended test species. A good laboratory practice statement was not included.

Table I 6. Summary of freshwater invertebrate acute data from EPA's ECOTOX database and other open literature studies. No values were lower than the lowest submitted value of 101.2 µg ai/L.

Genus	Species	Common Name	Dur (days)	LC_{50} value (95% C.I.) µg/L	Exp Type	Ecotox Ref #	Comment
Corbicula	*manilensis*	Asiatic clam	2	20000	S/U	5016	
Orconectes	*nais*	Crayfish	4	2000	S/U	15172	
Macrobrachium	*rosenbergii*	Giant river prawn	2	3470	S/U	5016	
Procambarus	*clarkii*	Red swamp crayfish	4	200	R	11621	
Procambarus	*clarkii*	Red swamp crayfish	4	200	S/U	11621	
Procambarus	*clarkii*	Red swamp crayfish	4	6500	S/U	15172	
Brachionus	*calyciflorus*	Rotifer	1	6500	S/U	5096	
Brachionus	*calyciflorus*	Rotifer	1	47820	S/U	61819	
Gammarus	*pseudolimnaeus*	Scud	4	1000	S/U	15172	

Abbreviations; S=static; U=unmeasured

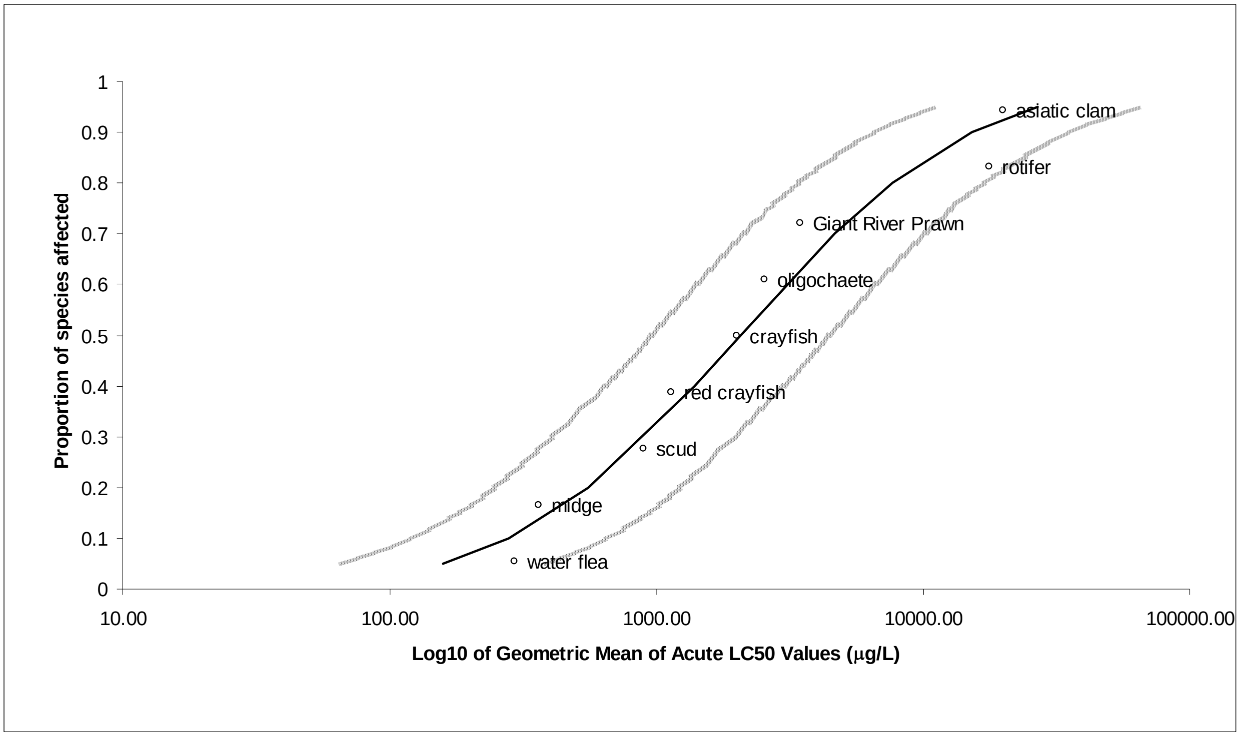

Figure I 2. Species sensitivity distribution on the acute toxicity of thiobencarb (TEP and TGAI) to freshwater invertebrates using all data accepted from the ECOTOX database and submitted studies.[3]

2.4. Freshwater Invertebrate, Chronic

An aquatic invertebrate life-cycle test using *Daphnia magna* using the TGAI is required for thiobencarb because the end-use product may be applied directly to water or expected to be transported to water from the primary use site (rice) and because the following conditions are met: (1) some aquatic acute LC_{50}'s and EC_{50}'s are less than 1 mg/l, (2) EECs in water (based on measured concentrations) were greater than 1% of acute LC_{50} and EC_{50} values, and (3) the half-life in water is greater than 4 days. *Daphnia magna* is the preferred test species.

[3] This species sensitivity distribution was developed using the Species Sensitivity Generator version 1.0 available from Causal Analysis/Diagnosis Decision Information System (CADDIS).

Table I 7. Summary of chronic toxicity studies for freshwater invertebrates

Taxon TGAI/TEP Test System Study type/ nominal or measured	Duration and Endpoint (95% C.I.)	MRID or Source and Study Classification DER Date	Comments
Water flea *Daphnia magna* TGAI 96.9% Flow-through/ measured	Life-cycle 21-day NOAEL/ LOAEL = 48/ 90 µg ai/L (offspring produced)	MRID 42680401 Supplemental 05/10/1993	Treatments: 23, 48, 90, 180, 390 µg ai/L mean measured. Solvent used in stock solution preparation was changed from TEG to acetone from day 7 to test termination for the exposure solutions and from day 13 to test termination for the solvent control solution. The solvent switch did not appear to affect the results. In addition, chemical analysis was not performed immediately after the switch.
Water flea *Daphnia magna* TGAI 95.2-95.9% Flow-through/ measured	21-day NOAEC/LOAEC = 1.0/ 3.0 µg ai/L (offspring produced)	MRID 00079098 Acceptable 07/23/1980	Treatments: 0.035, 0.016, 0.006, 0.003, 0.001 mg/L
Chironomus Riparius TGAI 97.2% Static-sediment/ measured	28-day NOAEC/ LOAEC = 0.18/ 0.42 mg/L overlying water TWA concentration (percent emergence, sex ratio)	MRID 46091402 Preliminary Supplemental	Treatments: 0.45, 1.04, 2.15, 4.56, 9.95 mg/L TWA in overlying water, total recovery was 70-33% of nominal. Aerated. Possible loss via volatization. Measured total radioactivity without identity confirmation. Emergence did not occurr day 15-28 in controls and should have occurred between day 12 -23.

Toxicity of thiobencarb (95.2-95.9% ai) was examined in a life-cycle toxicity study for daphnid, *Daphnia magna*. The NOAEC and LOAEC were 1.0 µg ai/L and 3.0 µg ai/L, respectively. Chronic effects observed were reduced number of young produced and adult mortality. These results indicate that concentrations of thiobencarb greater than 1 µg ai/L can be detrimental to the survival and reproduction of freshwater invertebrates. Additionally, a supplemental sediment toxicity test showed that sediment toxicity (decreased percent emergence and altered sex ratio) occurred when the time weighted average concentration of thiobencarb in overlying water was greater than 180 µg/L. No open literature chronic toxicity studies were available for freshwater invertebrates in the acceptable ECOTOX report.

2.5. Estuarine and Marine Vertebrates, Acute

Acute toxicity testing with estuarine and marine organisms (fish, shrimp, and oysters) using the TGAI is required when an end-use product is intended for direct application to the marine/estuarine environment or is expected to reach this environment in significant concentrations. The preferred test organisms are the sheepshead minnow, mysid shrimp and eastern oyster. Estuarine/marine acute toxicity testing is required for this pesticide because its use on rice is expected to result in significant exposure to marine and estuarine environments. Application of thiobencarb on rice fields will contaminate tail water (*i.e.*, water discharged from the water management system) which may flow into estuaries. Table I 8 through Table I 10 show the results of these tests for estuarine/marine fish.

Table I 8. Summary of submitted acute toxicity studies to estuarine/marine fish

Taxon TGAI/TEP Test System Study type/ nominal or measured	**Duration and Endpoint (95% C.I.)**	**MRID or Source and Study Classification DER Date**	**Comments**
Sheepshead minnow			
Sheepshead minnow *Cyprinodon variegatus* TGAI 95.1% Flow-through/ measured	96-hr LC_{50} = 660 (600-800) µg ai/L	MRID 00079112 Acceptable 01/17/1980	Treatments: 260, 390, 580, 1300, 2200 µg ai/L mean measured. 41-55% of nominal
Sheepshead minnow *Cyprinodon variegatus* TGAI 95.1% Static	96-hr LC_{50} = 900 (700-1200) µg ai/L	MRID 00079110 Acceptable 01/16/1980	Treatment: 0.4, 0.6, 1.1, 1.8, 3.0 µg ai/L
Sheepshead minnow *Cyprinodon variegatus* TEP 85.5% Static	96-hr LC_{50} = 1400 (1100-1800) µg ai/L	MRID 00079111 Acceptable 01/17/1980	Formulation Bolero 8 EC Treatments: 0.4, 0.6, 1.1, 1.8, 3.0 µg ai/L

The results indicate that thiobencarb is highly toxic to marine/estuarine fish on an acute basis. Sheepshead minnow were more sensitive to the TGAI than the TEP. The 96-hr LC_{50} for the TGAI was 660 (600-800) and 900 (700-1200) µg ai/L while the value for the TEP was 96-hr LC_{50} = 1400 (1100-1800) µg ai/L (MRID 00079112, 00079110, 00079111). The lowest value for the TGAI at 660 µg ai/L. Thiobencarb and thiobencarb formulations are moderately to highly toxic to estuarine/marine fish. The estuarine/marine fish acute toxicity testing requirement using technical end product and TGAI (Guideline 72-1) is fulfilled.

Additional Open Literature Information (ECOTOX) for Freshwater Fish Acute Values

All of the open literature acute values from EPA's ECOTOX database for estuarine/marine fish are presented in Figure I 3. These data are primarily for an exposure duration of 4 days; however, other durations were included (*e.g.*, 1 or 2 day exposures) if there was no 4 day LC_{50} for a particular test. Table I 10 contains the acute LC_{50} values that are less than the benchmark of 660 µg ai/L. When LC_{50} values for multiple exposure durations from the same test were available, only the value for 4 day exposure was included. There were 25 LC_{50} values from two open literature studies representing four estuarine/marine fish species. Almost all of these values were below the estuarine/marine fish benchmark (660 µg/L) that was based on the information provided by the registrant. The lowest of these more sensitive acute LC_{50} values is 204 µg a.i./L for the Atlantic silverside, *Minidia menidia* (Borthwick *et al.*, 1985). This study was evaluated and considered quantitative.

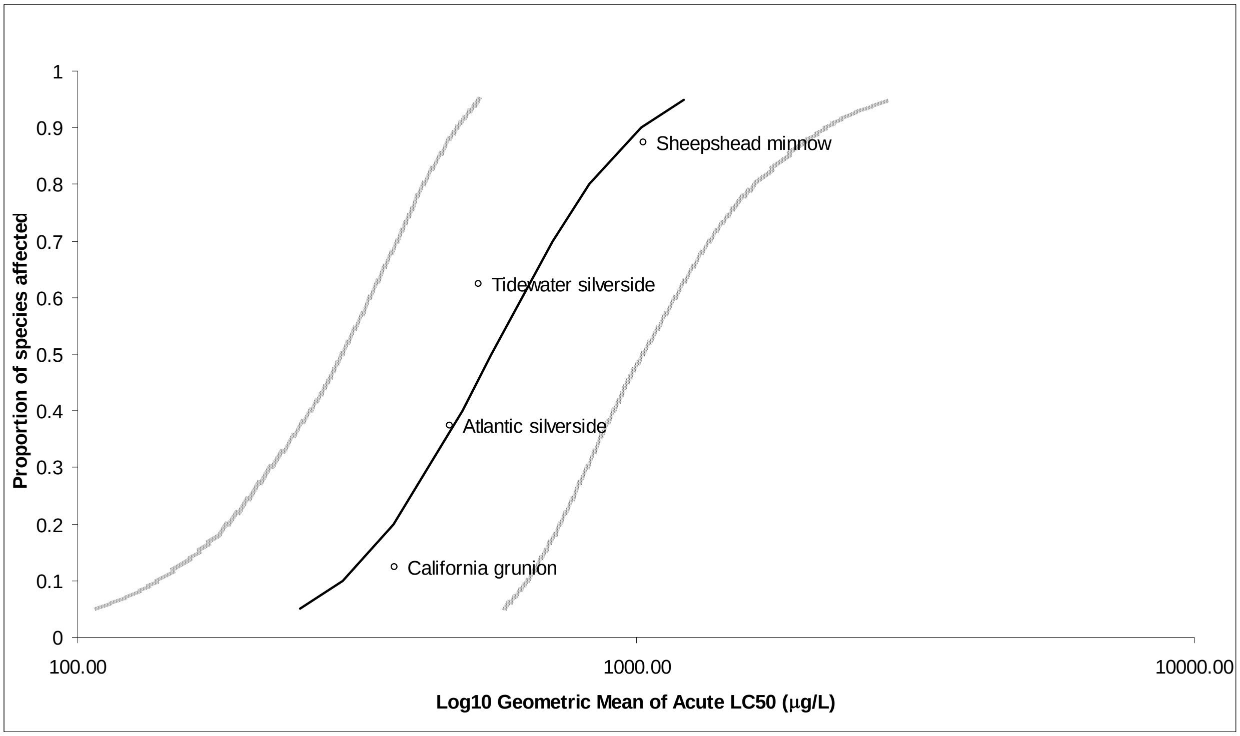

Figure I 3. Species sensitivity distribution on the acute toxicity of thiobencarb (TEP and TGAI) to estuarine/marine fish using all data accepted in the ECOTOX database and submitted studies.[4]

Table I 9. Summary of estuarine/marine fish acute data from EPA's ECOTOX database and other open literature studies.

Species **Test Method**	**Study Type**	**96-hr LC_{50} in µg ai/L (95% Confidence Interval) – age**	**ECOTOX Reference number and comments**
California grunion *Leuresthes tenuis*	Static	96-hr LC_{50} = 309 (248-380) - 0 days old	E11868 Supplemental-

[4] This species sensitivity distribution was developed using the Species Sensitivity Generator verstion 1.0 available from Causal Analysis/Diagnosis Decision Information System (CADDIS).

		483 (378-617) - 7 days old 586 (448-738) - 14 days old 502 (408-618) - 28 days old*	quantitative
California grunion *Leuresthes tenuis*	Flow-through	96-hr LC_{50} = 265 (250-283) - 0 days old 237 (204-275) - 7 days old 377 (324-456) - 14 days old 333 (270-405 - 28 days old	E11868 Supplemental- quantitative
Atlantic silverside *Menidia menidia*	Static	96-hr LC_{50} = 458 (341-640) - 0 days old 450 (347-579) - 7 days old 633 (502-813) - 14 days old 748 (542-876) - 28 days old	E11868 Supplemental- quantitative
Atlantic silverside *Menidia menidia*	Flow-through	96-hr LC_{50} = 386 (304-457) - 0 days old 204 (177-242) - 7 days old 414 (350-493) - 14 days old 675 (603-781) - 28 days old	E11868 Supplemental- quantitative
Tidewater silverside *Media peninsulae*	Static	96-hr LC_{50} = 526 (442-645) - 0 days old 396 (270-540) - 7 days old 511 (414-632) - 14 days old 1174 (900-1621) - 28 days old	E11868 Supplemental- quantitative
Tidewater silverside *Media peninsulae*	Flow-through	96-hr LC_{50} = 299 (230-370) - 0 days old 464 (398-574) - 7 days old 394 (304-585) - 14 days old 817 (701-1056) - 28 days old	E11868 Supplemental- quantitative
Sheepshead minnow	Flow-through	1370	E15639

*Adjusted for control mortality of 10%.
*E11868 was also submitted to the EFED under MRID 00141967 and previously determined to be supplemental (Davy, 2008).

2.6. Estuarine and Marine Vertebrates, Chronic

Data from estuarine/marine fish early life-stage and aquatic invertebrate life-cycle toxicity tests are required if the product is applied directly to the estuarine/marine environment or expected to be transported to this environment from the intended use site, and when any one of the following conditions exist: (1) the pesticide is intended for use such that its presence in water is likely to be continuous or recurrent regardless of toxicity; (2) any acute LC_{50} or EC_{50} is less than 1 mg/L; (3) the EEC in water is equal to or greater than 1% of any acute EC_{50} or LC_{50} value; or (4) the actual or estimated environmental concentration in water resulting from use is less than 0.01 of any acute EC_{50} or LC_{50} value *and* any of the following conditions exist: studies of other organisms indicate the reproductive physiology of fish and/or invertebrates may be affected, physicochemical properties indicate cumulative effects, or the pesticide has a half-life in water greater than 4 days. The preferred test organisms are the sheepshead minnow and mysid shrimp.

Chronic testing with thiobencarb is required because it has a primary use (rice) for which it is applied directly to water or is applied to land which is subsequently flooded with water. In addition, concentrations of thiobencarb measured in aquatic field studies are as great as 0.085 mg/L, which is greater than 0.01 of the LC_{50} for marine/estuarine fish and aquatic invertebrates. Results of this test are given in Table I 10.

Table I 10. Summary of submitted chronic toxicity data for estuarine/marine species

Taxon TGAI/TEP Test System Study type/ nominal or measured	**Duration and Endpoint (95% C.I.)**	**MRID or Source and Study Classification DER Date**	**Comments**
Sheepshead minnow *Cyprinodon variegatus* TGAI 95.1% Flow-through/ measured	Early Life Stage 28-day post hatch NOAEC/ LOAEC <150/ 150 µg ai/L (wet weight) NOAECL/LOAEC 370/ 600 µg ai/L (hatching success) NOAEC/ LOEAC 150/ 230 µg ai/L (mortality)	MRID 00079112 Supplemental 01/17/1980	Treatments: 150, 230, 370, 600, 960 µg ai/L mean measured. 68-83% of nominal Low DO at many test levels. It does not appear that there was any relationship between the low dissolved oxygen levels and the results. Although there are significant differences (p & 0.01) between the DO levels for the seawater control and solvent control during the hatching period and 28-day juvenile study, there are no significant differences (p of 0.05) between the two control groups regarding any of the biological results. Low DO levels are believed to be the result of growth of bacteria that was enhanced by the presence of acetone. No survivors at highest test level and only 1 fish survived at 600 µg ai/L.

The results indicate that a concentration of 150 µg ai/L can adversely affect the growth of juvenile fish. Effects were observed at all treatment levels. Additionally, reduced survival and hatching success were observed at 230 and 600 µg ai/L. No chronic estuarine/marine vertebrate data were available in the acceptable ECOTOX report.

2.7. Estuarine and Marine Invertebrates, Acute

Table I 11 summarizes results from submitted toxicity studies for thiobencarnb on estuarine/marine invertebrates. The results indicate that thiobencarb is very highly toxic to highly toxic to shrimp species and very highly toxic to estuarine/marine mollusks on an acute basis. The results indicate that thiobencarb is also moderately toxic to the fiddler crab. An eastern oyster shell deposition study is not available.

The most sensitive estuarine/marine aquatic invertebrate tested is the mysid shrimp (*Mysidopsis bahia*) (96-hr LC_{50} = 96-hr LC_{50} = 150, 95% C.I. = 110-200 µg ai/L) (MRID 00050667). Other estuarine/marine invertebrates have similar sensitivity to less sensitivity to thiobencarb when compared to the mysid shrimp. All 96-hr LC_{50} values for all shrimp and Eastern oyster species ranged from 150 – 1100 µg ai/L**.** The fiddler crab was less sensitive than other species with a 96-hr LC50 value of 4400 µg/L.

One open literature study resulted in a lower endpoint than those observed in submitted studies (ECOTOX reference number E90259). The endpoint is not useable in the risk assessment due to incomplete reporting of test procedures and other major limitations of the study. All other acute endpoints in the acceptable ECOTOX report were higher than 150 µg/L.

Table I 11. Summary of acute toxicity studies for estuarine/marine invertebrates

Taxon TGAI/TEP Test System Study type/ nominal or measured	**Duration and Endpoint (95% C.I.)**	**MRID or Source and Study Classification DER Date**	**Comments**
Mysid Shrimp			
Mysid shrimp (< 1day old) *Mysidopsis bahia* TGAI 94.6% Static/ nominal	96-hr LC_{50} = 150 (110-200) µg ai/L	MRID 00050667 Acceptable 02/02/1980	Treatments: 38, 75, 150, 300, 600 µg ai/L
Mysid shrimp (6-8 days old) *Mysidopsis bahia* TGAI 95.1% Flow-through/ measured	96-hr LC_{50} = 288 (237-356) µg ai/L	MRID 79117 and 43031701 Supplemental 04/05/1995 07/18/1996	Treatments: 40, 94, 177, 235, 510 µg ai/L Test mysid were much older than recommended by *the* guidelines.
Mysid shrimp Mysidopsis bahia (< 1 day old)	96-hr LC_{50} = 330 µg ai/L	MRID 00141967 Supplemental (Davy, 2008)	Open literatures study
Shrimp			

Taxon TGAI/TEP Test System Study type/ nominal or measured	**Duration and Endpoint (95% C.I.)**	**MRID or Source and Study Classification DER Date**	**Comments**
Grass shrimp *Palaemonetes pugio*	96-hr LC_{50} = 1000 µg ai/L (adults) 96-hr LC_{50} = 380 - 570 µg ai/L (juveniles)	MRID 00080858 Supplemental 08/04/1980	Precipitate formed
White shrimp *Penaeus setiferus*	96-hr LC_{50} = 310 µg ai/L	MRID 00080858 Supplemental 08/04/1980	Precipitate formed
Pink Shrimp *Penaeus duorarum*	96-hr LC_{50} = 570 µg ai/L	MRID 00080858 Supplemental 08/04/1980	Precipitate formed
Brown shrimp *Penaeus azetecus*	96-hr LC_{50} = 470 µg ai/L	MRID 00080858 Supplemental 08/04/1980	Precipitate formed
Ghost shrimp	96-hr LC_{50} = 1100 µg ai/L	MRID 00080858 Supplemental 08/04/1980	Precipitate formed
Eastern oyster			
Eastern oyster *Crassostrea virginica* TGAI 95.1% Static/ nominal	48-hr EC_{50} = 560 (200-1300) µg ai/L (normal embryo larvae)	MRID 00079114 Acceptable 02/06/1980	
Eastern oyster *Crassostrea virginica* TEP 85.5% Static/ nominal	48-hr LC_{50} = 320 (200-510) µg ai/L (normal embryo larvae)	MRID 00079115 Supplemental 02/07/1980 12/05/1990	Formulation Bolero 8 EC
Other Species			
Fidler Crab *Uca pugilator* TEP 85.5% Static/ nominal	96-hr LC_{50} = 4400 (3500 – 5800) µg ai/L	MRID 00079113 Supplemental 02/06/1980	Treatments: 0.9, 1.6, 2.7, 4.6, 7.5 mg/L Not a recommended test species.

2.8. Estuarine and Marine Invertebrates, Chronic

Table I 11 summarizes results from submitted chronic toxicity studies for thiobencarb on estuarine/marine invertebrates. The results indicate that thiobencarb is very highly toxic to highly toxic to shrimp species and very highly toxic to estuarine/marine mollusks on an acute basis. The results indicate that thiobencarb is also moderately toxic to the fiddler crab.

The results indicate that a concentration of 6.2 µg ai/L can adversely affect the growth of estuarine invertebrates. However, there are some uncertainties in the studies cited in that none of the estuarine invertebrates studies were done in accordance with agency guidelines. Because the use of thiobencarb on rice in the Gulf Coast region may affect estuarine crustaceans, including economically important shrimp, these data on the chronic effects of thiobencarb on shrimp is essential for the risk assessment. Concentrations lower than 21 µg ai/L may result in mortality of grass shrimp.

Table I 12. Summary of Submitted chronic toxicity data for estuarine/marine species

Taxon TGAI/TEP Test System Study type/ nominal or measured	**Duration and Endpoint (95% C.I.)**	**MRID or Source and Study Classification DER Date**	**Comments**
Mysid shrimp *Mysidopsis bahia* TGAI 95.1% Flow-through/ measured	LOAEC = 11 µg ai/L (reproduction)	MRID 79117 and 43031701 Supplemental 04/05/1995 07/18/1996	Treatments: 11, 19, 30, 50, 96 µg ai/L mean measured The NOAEC could not be determined because the control had no replication.
Opossum Shrimp *Neomysis mercedis* TGAI	NOAEC/ LOAEC = 3.2/6.2 µg ai/L (survival of offspring)	MRID 43976801 or 40651314 Supplemental, Quantitative (USEPA, 1997)	Open literature study (MRID 43976801) that is referenced as supplemental in the RED. Data were submitted by the same author under MRID 40651314. No DER was completed. An open literature has been completed. Gravid females were replaced if they died for the first fourteen days. Replacement was not reported.
Grass shrimp *Palaemonetes pugio* TEP 84.7% Flow-through/ measured	NOAEL/LOAEL <21/ 21 µg ai/L (adult mortality)	MRID 00079097 Supplemental 07/14/1980	Treatments: 22, 21, 36, 52, 105, 218 µg ai/L, 42-70% of nominal Levels are highly uncertain because measured concentrations were highly variable.

3. Aquatic Field Studies

The conclusion of high risk to aquatic organisms, based on results from laboratory toxicity tests, triggered the requirement for aquatic field testing with thiobencarb (GLN 72-7). The following aquatic field studies have been conducted on the use of thiobencarb on rice.

Table I 13. Summary of submitted aquatic field studies on the use of thiobencarb on rice

Title	Location and Date	Reference	Performed By	Sponsor	Fulfills Guideline Requirements?
Studies in Halls Bayou to Test the Effects of a Pre-Emergent Herbicide, Bolero, on Aquatic Organisms	Halls Bayou/ Chocolate Bay, Brazonia County, Texas 1979	MRID 00079986	Harper, 1979	Chevron Chemical Company	No, supplemental
Impact of Bolero Runoff on a Brackish Water Ecosystem	Matagorda, Texas 1982 - 1984	MRIDs 42130705 & 42130708	Fujie, 1983.	Chevron Chemical Company	Yes[1]
Thiobencarb: Studies on Residue Level and Behavior in Selected Irrigation Creeks in Agricultural Areas in Saga Prefecture, Southwestern Japan	Saga Prefecture, Kyushu, Japan 1975	MRID 00028183	Ishikawa, 1975	Unknown	No, supplemental

[1] Following the review of this study, an additional aquatic field study was requested to monitor aquatic residues in other localities where rice is grown. This additional study, however, was waived in December 1993. No further field studies are requested for thiobencarb at this time.

Hall's Bayou Study: The first field study conducted in the U.S., was in rice fields bordering Halls Bayou, a tidally influenced, narrow stream that empties into West Bay near Galveston, Texas. This study is also referred to as the Chocolate Bay study. This estuarine area is a complex and highly important ecosystem that supports many commercial species. Contaminated water was released into the bayou when rice fields were irrigated with a small amount of water (*i.e.*, flushed) to moisten the soil. Also, heavy rainfall occurring during the experiment resulted in two additional releases of contaminated water. Sampling sites were established 500 ft downstream and 500 ft upstream of the point of discharge from the rice fields. Water samples collected at the field outlets and in Halls Bayou were analyzed for residues of thiobencarb. Fish, nektonic macroinvertebrates, benthic organisms, and phytoplankton were also sampled in these areas before, during, and after discharge from the rice fields. Fish and macroinvertebrates were also held in cages in Halls Bayou to monitor their response to the discharge of thiobencarb.

Due to poor experimental design and experimental conditions that caused excessive stress to the caged organisms, the EFED concluded that the results of the caged tests with fish and shrimp were invalid. They thus yield no information which can be used for risk assessment. Other parts of the field study provided some information and were classified as supplemental.

The highest concentrations of thiobencarb were measured on a day when heavy rainfall (3.23 inches) occurred on the same day that thiobencarb was applied, resulting in an unscheduled flush overflow. Peak thiobencarb concentrations were 8.9 mg/L (8900 µg ai/L) where the tail water exited the rice field and 690 ppb at the point where the drainage water entered Halls Bayou. The highest concentrations measured in the Halls Bayou on days that were not associated with heavy rainfall were 83 ppb at the upstream station (E) and 64 ppb at the downstream station (F). The abundance of fish, invertebrates, and plankton sampled at the downstream station were similar to or greater than those sampled at the upstream station. Gillnet catches declined in only one of the two areas sampled after discharges from the rice fields. Seine and trawl sampling indicated a decline in abundance of fish and invertebrates occurred near the end of the study. All declines were observed at both the upstream and downstream stations. Some differences in species composition of fish and invertebrates were observed between the upstream and downstream stations, and some changes in the species composition of benthic organisms were observed over time. None of these differences, however, could be conclusively linked to the discharge of thiobencarb.

The biological findings of the Halls Bayou study were inconclusive since there were no significant differences in species abundance or clear trends in the changes in species composition between stations upstream and downstream of the point of discharge. The upstream stations, being only 500 feet upstream of the site of discharge, were likely close enough to be affected by contamination moving upstream as the result of tidal mixing. Also, the abundance and composition of species were probably influenced by other factors, including tidal cycles, salinity changes, and release of other pesticides from neighboring areas. Small samples sizes further limited the usefulness of this study. This study does not provide much useful information on the effects of thiobencarb on the estuarine environment.

Matagorda Study: A larger aquatic field study was conducted in 1982-1984 near Matagorda, Texas. The site consisted of a rice field that drained through a ditch into the tidal waters of the lower Colorado River of eastern Texas. As with Hall's Bayou, this estuarine area is a complex and highly important ecosystem that supports many commercial species. No thiobencarb applications were made in 1982; this year provided baseline data for the site. Baseline thiobencarb concentrations were as high as 9 µg ai/L. In 1983 and 1984, approximately 500 acres of the field were treated with thiobencarb at a rate of 4 lbs ai per acre. Fields were flushed with water within 3 to 12 days after application. Data collected from 1982 through 1984 included (1) residues of thiobencarb in water, sediment, fish and shrimp; (2) catch per unit effort measurements of fish and aquatic invertebrates; and (3) percentages of grass shrimp (*Palaemonetes pugio*) that were gravid. While samples were collected during all three years of the study, the sampling effort on the third year was very poor.

A control station was also planned on the Colorado River upstream of the confluence with the drainage ditch. However, during the course of the study, the Agency and the registrants agreed that this station could not serve as a control for the field study because it contained preexisting residues of thiobencarb. It was therefore only possible to compare residues and biological samples collected during 1983 and 1984 to those collected during 1982, before the initial treatment. This represents a shortcoming of this study since the results could have been influenced by yearly fluctuations in environmental conditions that are unrelated to the applications of thiobencarb. Another shortcoming is that other pesticides (ordram, basegran, machette, and propanil) were applied to fields that drain into the test ditch during the period of this study. The toxicity of these pesticides could have contributed to the observed effects.

The results of the study were:

1. Residues of thiobencarb were transported into the estuary via runoff and drift. Residues in water exceeded the aquatic invertebrate Maximum Allowable Toxicant Concentration (MATC; 1.7 µg ai/L). Maximum residues measured in water, sediment, fish, and shrimp were 25.1 ppb, 50 ppb, 2400 ppb, and 970 ppb, respectively.

2. Although the overall population of fish was apparently not affected, marked declines were observed during the treatment years in three species, *Gambusia affinis, Dormitator maculatus,* and *Poecilia latipenna*.

3. Several taxa of aquatic invertebrates showed substantial decline in numbers caught per unit effort. Species richness and diversity also declined significantly during treatment years.

4. The percentage of gravid shrimp decreased significantly in 1983 compared to 1982. The decline was about 50% at stations 1 and 2, and averaged 23% for all four stations. Sampling was inadequate to assess the effect on the percentage of gravid shrimp in 1984.

5. A kill of the fish menhaden (*Brevoortia patronus*) was observed in the area where the field runoff entered the drainage ditch. It occurred at the point of discharge from the drainage canal, one to two days after a post-application flush of the rice fields. Although other pesticides that were applied that year (ordram, basegran, and propanil) may have been present in the tailwater, this kill was attributed to thiobencarb contamination because the dead fish contained high residues of thiobencarb (mean of 3.56 ppm).

6. Field BCF for thiobencarb was estimated to be 109X for fish and 44X for shrimp.

Declines in fish, aquatic invertebrates, and gravid shrimp cannot conclusively be attributed to the use of thiobencarb. Nevertheless, the findings in the field were consistent with effects demonstrated in laboratory studies. They suggest that the application of thiobencarb to rice fields may result in significant environmental damage to the adjacent estuarine habitat. Possible effects include chronic effects to sensitive fish, acute and chronic effects to ecologically important aquatic invertebrates, chronic effects to grass shrimp and possibly to commercial shrimp, and indirect detrimental effects to organisms at higher trophic levels that depend on these organisms for food.

Japan Study: The EFED reviewed a study that measured residues of thiobencarb in creek water after application to rice paddies in Japan. Thiobencarb was applied in the form of 7% granules at a rate of 30 kg/ha, which is equivalent to 1.9 lb ai/A. Water samples were taken from ten stations along creeks that flow through the rice fields and drain into the Hayatsue River. Water sampling was conducted from March through November, with thiobencarb treatments being made from June 28 through July 2. The creeks served as storage for irrigation water until May, when the water is pumped onto the fields. The creeks resembled large ponds during the storage period.

Very low thiobencarb concentrations (0.2 µg ai/L or less) were reported at all stations in March and April before applications were made. Concentrations peaked at the sampling period of July 1, when concentrations at most stations were between 20 and 40 µg ai/L. The greatest concentration was measured was 40.5 µg ai/L. Concentrations declined fairly rapidly thereafter; the half-life of thiobencarb in creek water was estimated to be 8.8 days. This rate of decline represents dilution as well as biological and physical degradation processes. EFED cannot interpret the significance of these results or extrapolate conclusions to other areas because of the lack of important information on the test conditions, such as flow rates within the creeks and rainfall during the study.

A difficulty with all three of the field studies was that water flow measurements were not made, making it impossible to discern effects of dissipation versus dilution. While water residues were generally short-lived, it is not clear whether thiobencarb residues were broken down by chemical or biological forces, or they were swept away and diluted by tidal flow. Because it is possible that dilution was the primary mode of dissipation in all three studies, the rate at which thiobencarb degrades by chemical or biological means in estuaries remains unknown. Thiobencarb residues thus may persist longer in other areas where dilution is of less importance in the dissipation of residues.

The three biological field studies demonstrate that application of thiobencarb on rice can cause significant contamination to water, sediments, and aquatic organisms in off-site aquatic habitats. Harm to estuarine and freshwater ecosystems is possible when thiobencarb is used in the southeastern United States. Although shortcomings of these studies make it impossible to identify thiobencarb as the sole cause of observed adverse effects, the studies fail to refute the Agency's presumption that the use of thiobencarb on rice results in severe effects on aquatic ecosystems.

4. Toxicity to Terrestrial Animals

4.1. Birds, Acute and Subacute

An oral (LD_{50}) study (preferably mallard or bobwhite quail and a passerine species) and two subacute dietary (LC_{50}) studies (one species of waterfowl, preferably the mallard duck and one species of upland game bird, preferably bobwhite quail) are required to establish the toxicity of a pesticide to birds. Results of these tests are shown in Table I 14. Acute toxicity data on a passerine species were not submitted, thus avian acute oral toxicity to songbirds is an uncertainty.

Table I 14. Summary of acute and subacute toxicity studies for avian species

Taxon TGAI/TEP Measured/ nominal	Duration and Endpoint (95% C.I.)	MRID or Source Classification DER Date	Comments
Bobwhite quail *Colinus virginianus* TGAI 96.9% nominal	Acute oral LD_{50} > 1938 mg ai/kg-bw NOAEL = 969 mg ai/kg-bw	MRID 42600201 Acceptable 08/22/1995	Treatments: 60.6, 121, 242, 485, 969, 1938 mg ai/kg-bw Capsule Slight decrease in bw observed during

Taxon TGAI/TEP Measured/ nominal	**Duration and Endpoint (95% C.I.)**	**MRID or Source Classification DER Date**	**Comments**
			first 3 days after dosing. No mortality observed at 1938 mg ai/kg-bw
Mallard duck *Anas platyrhynchos* TGAI %96.5 nominal	5-day LC_{50} >5000 mg ai/kg-diet NOAEC = 648 mg ai/kg-bw (body weight gain)	MRID 44846206 Acceptable 10/06/2002	Treatments: 648, 1080, 1800, 3000, 5000 mg ai/kg-diet No treatment related mortality. Sublethal: increased water consumption, lack of coordination, smaller appearance, decreased bw gain (57-10% in treatments versus 76-77% in control), decreased food consumption, in ≥1080 mg ai/kg-diet treatment. Birds completely recovered after 28-days.
Bobwhite quail *Colinus virginianus* TGAI/ NS		MRID 00025774 Supplemental 05/25/1980	Treatments: 562, 1000, 1780, 3160, 5620 mg ai/kg-diet Percent ai not specified. Brain cholinesterase activity significantly different than control. Decreased weight gain, decreased egg production were also observed.

These results indicate that thiobencarb is practically nontoxic to avian species on an acute oral basis. An acceptable study (MRID 44846206) suggests that thiobencarb is probably practically nontoxic to the bobwhite on a subacute dietary basis. A subacute dietary study with an upland game species is still needed.

No acceptable data were available in the ECOTOX database examining acute toxicity of thiobencarb to birds.

4.2. Birds, Chronic

Avian reproduction studies using the technical grade of the active ingredient (TGAI) are required when birds may be exposed to a pesticide repeatedly or continuously through its persistence, bioaccumulation, or from multiple applications, or if mammalian reproduction tests indicate possible adverse reproductive effects. The preferred test species are the mallard duck and bobwhite quail. Avian reproduction studies are required for thiobencarb because it is persistent in the terrestrial environment and may bioaccumulate. Results of these tests are given in Table I 15. The results indicate that dietary concentrations greater than 100 mg ai/kg-diet can impair reproduction in birds.

Table I 15. Summary of chronic avian studies.

Taxon TGAI/TEP Measured/ nominal	Duration and Endpoint (95% C.I.)	MRID or Source Classification DER Date	Comments
Mallard duck *Anas platyrhynchos* TGAI % 95.5	1-generation NOAEC/ LOAEC = 100/ 300 mg ai/kg-diet (eggs laid and hatchlings per live 3 week embryo)	MRID 00025778 Supplemental 08/05/1997	Treatments: 30, 100, 300, 1000 mg ai/kg-diet Raw data were not complete for hatching success, hatching weight, and 14-day survivor weight. Supplemental due to temperature and humidity not controlled; photoperiod increased very rapid and at very high photoperiod; raw data provided is incomplete; and housing and feed not described well. Sublethal Effects: depression, loss of coordination, lower limb weakness, prostrate posture, loss of reflexes, wing droop. Observed in 1 bird at 30 and 300 mg ai/kg-diet and 2 birds at 1000 mg ai/kg-bw. Dissapeared after 2 days.
Mallard duck *Anas platyrhynchos* TGAI 96.5% measured	Avian reproduction (27 weeks) NOAEL/LOAEL = 115/ 231 mg ai/kg-diet (14-day survivor weight) NOAEL/LOAEL = 231/ 338 mg ai/kg-diet (normal hatchings eggs laid, eggs set)	MRID 45140601	Treatments: 115, 231, and 338 mg ai/kg-diet mean measured Adult ducks were exposed to thiobencarb for only 12 days prior to egg production. The birds should be exposed for 10 weeks prior to egg production.
Japanese Quail *Coturnix japonica* TEP 50% ai	1-generation NOAEC/ LOAEC = 750/ 1000 mg ai/kg-drinking water (fertility and hatchability) NOAEC/ LOAEC = 1000/ 3750 mg ai/kg-drinking water (egg weight, eggs laid, amount solution consumed)	MRID 00080848 Supplemental 07/15/1980	Saturn 50% EC placed in drinking water available ad libitum Treatments:3.5, 35, 185, 350, 750 mg/L Supplemental due to lack of information on procedures, raw data, housing, consumption data, and test environment
Bobwhite quail *Colinus virginianus* TGAI 97.5% Mean measured	1-generation NOAEC/ LOAEC = 267/ 930 mg ai/kg-diet (% normal live embryos, weight of hatchlings) NOAEC >86.2 mg ai/kg-diet (eggs	MRID 43075401 Acceptable 07/07/1995	Treatments: 86.2, 267, 930 mg ai/kg-diet Decrease in body weight of birds in the 930 mg ai/kg-diet treatment group during the first two weeks of exposure. Apparent reductions in the number of hatchlings and number of 14-day old survivors per hen at 930 mg ai/kg-diet group but these parameters were not

Taxon TGAI/TEP Measured/ nominal	Duration and Endpoint (95% C.I.)	MRID or Source Classification DER Date	Comments
	cracked)		significantly different from the control group. Significant decrease in number of eggs cracked at 86.2 and 930 mg ai/kg-diet treatment levels.

4.3. Mammals

Wild mammal testing may be required on a case-by-case basis, depending on the results of the lower tier studies such as acute and subacute testing, intended use pattern and pertinent environmental fate characteristics. This testing has not been required for thiobencarb. Mammalian toxicity data reported by the Health Effects Division is available in Table I 16. Acute oral LD_{50} data for laboratory rats submitted to the Health Effects Division (HED) for evaluation of human toxicity were used to assess the mammalian acute toxicity of thiobencarb. The LD_{50} for male and female rats are 1033 and 1130 mg ai/kg, respectively (MRID 42130701). These results classify thiobencarb as slightly toxic to mammals on an acute basis.

Smith (1993)[5] reports that the LD_{50} of technical grade thiobencarb is 920-1903 mg/kg in the rat, which supports the definitive findings reported above. Smith also reports the LD_{50} of technical grade thiobencarb for the mouse to be 2745 mg/kg, indicating that the mouse is less sensitive than the rat (Smith, 1993).

In a combined chronic toxicity/carcinogenicity feeding study (MRID 00154506), Fischer 344 rats received 0, 20, 100 or 500 mg/kg-diet (approximately 0, 1, 5, and 25 mg/kg/day by standard conversion methods) technical thiobencarb (95.3% a.i.) in the diet for two years. Systemic toxicity was noted at 100 mg/kg-diet and above as decreased body weight gain, food consumption and food efficiency. There was also an increase in blood urea nitrogen. No evidence of carcinogenicity at the dose levels tested was observed. For chronic toxicity, the NOAEL was 1 mg/kg/day (20 mg/kg-diet) and the LOAEL was 5 mg/kg/day (100 mg/kg-diet) based on decreased body weight gains, food consumption, food efficiency, and increased blood urea nitrogen.

In a two generation reproduction study (MRID 40446201), Charles River CD rats received either 0, 2, 20, or 100 mg/kg/day technical thiobencarb (96.7% a.i.) by daily oral gavage in 0.5% CMC aqueous solution. Systemic toxicity was noted at 20 mg/kg/day and above based on enlargement of centrolobular hepatocytes (both generations) and hepatocyte single cell necrosis observed in both sexes of both generations including renal atrophic tubule consisting of regenerated epithelium. There were increased liver weights (absolute and relative) and increased kidney weights (absolute and relative) in the high dose group. There were also significant changes on

[5] Smith, G. J. 1993. Toxicology & Pesticide Use in relation to wildlife: Organophosphorus & Carbamate compounds. C. K. Smoley, Boca Raton, FL.

body weights at 100 mg/kg/day and male kidney weights were increased in the high dose group. There were no effects on reproductive parameters. For Parental/Systemic toxicity, the NOAEL was 2 mg/kg/day and the LOAEL was 20 mg/kg/day based on histopathological changes of the liver and kidney. For reproductive toxicity, the NOAEL was equal to or greater than 100 mg/kg/day and the LOAEL was greater than 100 mg/kg/day.

Thiobencarb was rapidly absorbed after oral administration with almost all eliminated in the urine within 72-hours (MRID 42340302).

Other sublethal effects observed in rats include gait abnormalities, decreased sensory responses, and decreased motor activity in an acute neurotoxicity screening at 500 mg/kg-day (NOAEL = 100 mg/kg-day, MRID 42987001, 43148202, acceptable).

Table I 16. Summary of mammalian toxicity data

Taxon TGAI/TEP Measured/ nominal	Duration and Endpoint (95% C.I.)	MRID or Source Classification DER Date	Comments
Rat TGAI 96%	Acute oral LD_{50} = 1033 (924-1155) mg ai/kg-bw males 1133 (1033-1247) mg ai/kg-bw male	MRID 42130701 (Lewis, 1997)	
Mouse	Acute oral LD_{50} = 2745 mg ai/kg-bw	(Smith, 1993)	
Fischer 344 rat	NOAEL/ LOAEL = 1/ 5 mg/kg/day or 20/ 100 mg/kg-diet (decreased body weight gain, food consumption, food efficiency, and increased blood urea nitrogen)	MRID 00154506	
Fischer 344 Rat	NOAEL/ LOAEL = 20/ 100 mg/kg/day (decreased body weight) 2-year NOAEL ≥ 100 mg/kg/day for reproductive effects	MRID 40446201 (Lewis, 1997)	

4.4. Terrestrial Invertebrates

Thiobencarb is considered practically nontoxic to honey bees (*Apis mellifera*) on an acute contact exposure basis (MRID 46059804). In this study, adult bees were exposed to a TGAI via oral and contact exposure routes at nominal concentrations of 0 and 100 µg a.i./bee. At the highest concentration tested in the oral test, 23.3% of the bees died in the treatment group as opposed to 6.7 and 3.3% in the negative and solvent control groups, respectively. In the contact exposure group, 15% of bees died as compared to 6.7 and 10% in the control groups. The 48-hr LD_{50} is thus greater than 100 µg ai/bee for both studies. No sublethal effects were observed. This study is classified as supplemental because the age of the bees at test initiation was not reported.

The acceptable ECOTOX data were examined for toxicity data using non-target species with endpoints expressed in terms similar to those for the standard test with honey bees. No values were available. Values were reported for the brown plant hopper; however, the value reported was for an aquatic test for a snail. Two studies were available for nematodes. The LOAEL reported for the Nemata and root-knot nematode were 0.75 kg ai/ha with the endpoint examined being population effects (Das *et al.*, 1997; Das *et al.*, 1998). These values were not reviewed for possible use in the calculation of a risk quotient.

Table I 17 . Summary of terrestrial insect toxicity data

Taxon TGAI/TEP Measured/ nominal	**Duration and Endpoint (95% C.I.)**	**MRID or Source Classification DER Date**	**Comments**
Honey Bee *Apis Mellifera* TGAI 97.2% Nominal	Acute oral LC_{50} >100 µg/bee Acute contact > 100 µg/bee	MRID 46059804 Acceptable	Contact: applied in acetone
Apple Snail *Pomacea aludosa* TEP 84% ai Static/NR in DER	96-hr LC_{50} = 1850 (1651-2072) µg ai/L	MRID 40031001 Acceptable 05/29/1987	Bolero 8EC The apple snail is terrestrial; however, the toxicity test was performed in water.
Cabbage Looper *Trichoplusia ni larvae* TGAI 93%	32-d LC_{50} = 298 (255-349) mg /kg-diet	E070313 (Brown, 1986)	Not reviewed

5. Toxicity to Plants

5.1. Terrestrial Plants

Terrestrial plant testing (seedling emergence and vegetative vigor) is required for herbicides which have terrestrial non-residential outdoor use patterns and which may move off the application site through volatilization (vapor pressure $\geq$1.0 x 10^{-5} mm Hg at 25^{o}C) or drift (aerial or irrigation), and/or which may have endangered or threatened plant species associated with the application site. Terrestrial plant testing is required for thiobencarb because it is an herbicide with a terrestrial nonresidential use pattern (rice) and because aerial applications may result in drift.

For the seedling emergence and vegetative vigor testing the following plant species and groups should be tested: (1) six species of at least four dicotyledonous families, one species of which is soybean (*Glycine max*), and another of which is a root crop, and (2) four species of at least two monocotyledonous families, one species of which is corn (*Zea mays*).

Results of Tier II seedling emergence toxicity testing on technical thiobencarb are given in Table I 18.

All open literature endpoints for terrestrial plants in the accepted ECOTOX data are higher than those measured in submitted studies. Efficacy studies were not included in the accepted ECOTOX data,

Table I 18. Summary of submitted terrestrial plant Tier II seedling emergence toxicity results for thiobencarb

Species	% a.i.	Parameter Affected	EC_{25} (lb ai/A)	NOAEC (lb ai/A)	MRID No. Author/Year
Monocot--Corn	96.6	Shoot length	>1.7	1.7	MRID 41690902 Hoberg, J.R. 1990
Monocot--Oat		Shoot length	0.086	0.055	
Monocot--Onion		Shoot length	2.0	0.94	
Monocot--Ryegrass		Mortality	0.019	0.0051[1]	
Dicot/Root Crop--Carrot		Shoot length	>3.1	2.1	
Dicot--Cabbage		Shoot length	0.082	0.071	
Dicot-Cucumber		Shoot length	>1.7	0.16	
Dicot--Lettuce		Mortality	0.27	--	
Dicot--Soybean		Shoot length	>1.7	0.94	
Dicot--Tomato		Shoot length	1.1	0.94	
Dicot - Lettuce Monocot – Ryegrass	96.5	None (measured seedling emergence, seedling survival, plant height, dry weight, and phytotoxicity)	> 0.010 lb ai/A.	>0.010 lb ai/A	MRID 44846201 Chetram, R.S. 1999 Acceptable DER 11/16/2002

1 This NOAEL is based on 17% mortality of plants occurring at the next higher test level, 0.011 lb ai/A.
2 Seedling emergence data for ryegrass is upgraded from supplemental to core.

In the tier II seedling emergence test, mortality of test plants occurred in the tests with ryegrass, cabbage, and lettuce. Mortality was the most sensitive toxic endpoint for these species (plants tended to die shortly after emerging). The most sensitive species was ryegrass, a monocot, for which the EC_{25} based on mortality (*i.e.*, LC_{25}) was 0.019 lb ai/A. The most sensitive dicot was cabbage. The cabbage EC_{25} based on shoot length was estimated to be 0.082 lb ai/A.

Results of Tier II seedling vegetative vigor toxicity testing on the technical thiobencarb are given in Table I 19.

Table I 19. Summary of submitted Tier II seedling vegetative vigor toxicity testing for thiobencarb

Species	% A.I.	Parameter Affected	EC_{25} (lb ai/A)	NOAEC (lb ai/A)	MRID No. Author/Year
Monocot- Corn	96.6	Shoot length,	>2.2	2.2	MRID 41690902

Species	% A.I.	Parameter Affected	EC_{25} (lb ai/A)	NOAEC (lb ai/A)	MRID No. Author/Year
		shoot weight, and root weight			Hoberg, J.R. 1990 Sup
Monocot--Oat		Shoot weight	0.17	0.12	
Monocot--Onion		Shoot length	1.2	0.80	
Monocot--Ryegrass		Shoot length	0.073	0.020	
Dicot/ Root Crop--Carrot		Shoot length, shoot weight, and root weight	>2.2	2.2	
Dicot--Cabbage		Root weight	1.2	1.4	
Dicot--Cucumber		Shoot weight and root weight	--[a]	<0.12	
Dicot--Lettuce		Root weight	1.3	0.80	
Dicot--Soybean		Shoot weight	1.2	0.80	
Dicot--Tomato		Root weight	1.8	2.2	

[a]Greater than a 25% reduction was recorded at some or all exposure levels, but the EC_{25} could not be determined because no dose-response relationship was apparent.

In the Tier II vegetative vigor tests, soybean and cucumber were the most sensitive dicot and ryegrass was the most sensitive monocot.

All open literature endpoints for terrestrial plants in the accepted ECOTOX data are higher than those measured in submitted studies.

5.2. Aquatic Plants

Aquatic plant testing is required for any herbicide which has outdoor non-residential terrestrial use in which it may move off-site by runoff (solubility >10 ppm in water), by drift (aerial or irrigation), or which is applied directly to aquatic use sites (except residential). The following species should be tested: *Kirchneria subcapitata*, *Lemna gibba*, *Skeletonema costatum*, *Anabaena flos-aquae*, and a freshwater diatom. Aquatic plant testing is required for thiobencarb because it may be applied directly to water, it may be applied aerially, and it is applied to rice paddies where it is expected to contaminate the tail water that leaves the field.

Results of Tier II toxicity testing on technical thiobencarb are given below.

Table I 20. Summary of submitted Tier II aquatic plant toxicity testing for thiobencarb

Species	Duration	EC_{50} (95% C.I.) µg ai/L	NOAEC µg ai/L	MRID No. Author/Year	Observed Effect
Freshwater diatom *Navicula pelliculosa* TGAI 96.6%	120 hrs	380 (240-610)	65	MRID 41690901 Giddings, J.M. 1990 DER 09/18/1995	Cell density
Duckweed *Lemna gibba* TGAI 96.6%	14 day	770 (380 – 1600)	140		Frond production
Green algae *Selenastrum capricornutum* TGAI 96.6%	120 hrs	17 (12 – 26)	13		Cell density
Marine diatom *Skeletonema costatum* TGAI 96.6%	120 hrs	73 (26 – 200)	18		Cell density
Blue-green algae *Anabaena flos-aquae* TGAI 96.6%	120 hrs	>3100	3100		Increased cell density at all concentrations
Marine diatom *Skeletonema costatum* TGAI 95.5%	96 hrs	327-459[a]	--	MRID 00141967 Borthwick and Walsh, 1981.	Open literature, supplemental

[a]96-hour EC_{50}

The Tier II results indicate that green algae is the most sensitive aquatic plant species. A thiobencarb concentration of 17 µg ai/L is predicted to result in a 50% reduction in cell density of green alga.

Two open literature studies are available with endpoints lower than those measured in submitted studies. The three day LOAEC for *Chlorella saccharophila* (a freshwater alga) was 3 µg ai/L (ECOTOX E15718) (Hirata *et al.*, 1984). This value may not be used because the solvent control was significantly different from the negative control and the solvent likely influenced results(Hirata *et al.*, 1984). Additionally, a 4-day NOAEC of 5 µg ai/L was reported for green algae (*Scenedesmus acutus*) with effects on cell density observed at 9 µg ai/L (ECOTOX E17114). The LC_{50} value was 4.0 mg/L and it could only be used qualitatively because the control growth rate could not be verified and the origin and possible contamination of the dilution water was not described (Sabater and Carrasco, 1996).

6. Invalid Studies

The following studies (identified by MRID number) were found to be invalid: 25779, 34763, 57222, 57223, 57224, 57225, 57227, 57228, 80852, 116143, 25783, 80853, 81906, 80854, 80856, 80857, 25774. Some were used in previous assessments.

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