

**Data Evaluation Report on the Acute Toxicity of AE 0317309 Technical to Fish,  
*Cyprinodon variegatus***

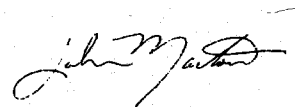
PMRA Submission Number 2006-2445

EPA MRID Number 468017-26

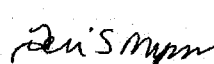
<b>Data Requirement:</b>	PMRA Data Code	9.5.2.4
	EPA DP Barcode	D328639
	OECD Data Point	IIA 8.2.1
	EPA MRID	468017-26
	EPA Guideline	850.1075 (72-3a)

**Test material:** AE 0317309 Technical **Purity:** 95.4%  
**Common name:** Pyrasulfotole  
**Chemical name:** IUPAC: Not reported  
CAS name: (5-Hydroxy-1,3-dimethyl-1H-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]methanone  
CAS No.: 365400-11-9  
Synonyms: None reported

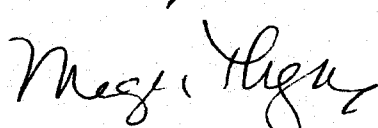
**Primary Reviewer:** John Marton  
Staff Scientist, Cambridge Environmental Inc.

**Signature:**   
**Date:** 5/09/06

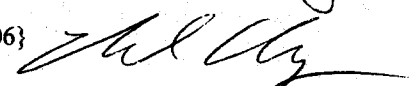
**Secondary Reviewer:** Teri S. Myers  
Senior Scientist, Cambridge Environmental Inc.

**Signature:**   
**Date:** 5/21/06

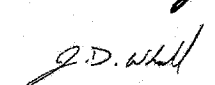
**Primary Reviewer:** Megan Thyne  
EPA

**Date:** 8/28/06 

**Secondary Reviewer(s):** Melissa Panger  
EPA

**Date:** 8/31/06 

**Secondary Reviewer(s):** J.D. Whall (Officer No. 1268)  
PMRA

**Date:** 11/23/06 

**Secondary Reviewer:** David McAdam  
Australian Government Department of the Environment and Heritage.

**Date:** 6 Nov 2006 

**Reference/Submission No.:** {.....}

**Company Code** BCZ  
**Active Code** PSA  
**Use Site Category:** 13, 14  
**EPA PC Code** 000692

**Date Evaluation Completed:** 12-05-2006

**CITATION:** Banman, C.S., M.E. Kern and C.V. Lam. 2004. Acute Toxicity of AE 0317309 Technical to the Sheepshead Minnow (*Cyprinodon variegates*) Under Static Conditions. Unpublished study performed by Bayer CropScience, Research and Development, Stilwell, KS. Laboratory study number EBAIX012. Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study completed May 24, 2004.

**DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to fish. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable



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data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

## **EXECUTIVE SUMMARY:**

In a 96-h acute toxicity study, Sheepshead Minnow (*Cyprinodon variegatus*) were exposed to AE 0317309 Technical at nominal concentrations of 0 (negative control) and 100 mg a.i./L under static conditions. Mean-measured concentrations were <9.92 (<LOQ; negative control) and 100 mg a.i./L. The 96-h LC<sub>50</sub> was >100 mg a.i./L. The EC<sub>50</sub> and NOAEC values, based on mortality and sub-lethal effects, were >100 and 100 mg a.i./L, respectively. No mortality or sub-lethal effects were observed in the negative control or in the mean-measured 100 mg a.i./L treatment level. Because the mean-measured concentration was 100 mg a.i./L after being corrected for purity of the active ingredient (95.4%), the reviewer cannot accurately assign a toxicity category to this chemical, as the test organisms were not exposed to concentrations of 100 mg a.i./L. Therefore, the reviewer can only say that AE 0317309 does not appear to be toxic to *Cyprinodon variegatus* at a concentration of 100 mg a.i./L.

This toxicity study is scientifically sound, classified as **ACCEPTABLE**, and does satisfy the guideline requirement for an acute toxicity study with Sheepshead minnow (*Cyprinodon variegatus*).

## **Results Synopsis**

Test Organism Size/Age (mean weight or length): 0.26 (0.17-0.48) g; 21.3 (19.0-26.0) mm; based on control fish at test termination

Test Type (Flow-through, Static, Static Renewal): Static

LC<sub>50</sub>: >100 mg a.i./L      95% C.I.: N/A

NOAEC: 100 mg a.i./L      Probit Slope: N/A

EC<sub>50</sub>: >100 mg a.i./L

Endpoint(s) Affected: None

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**I. MATERIALS AND METHODS**

**GUIDELINE FOLLOWED:** Procedures in this study followed guidelines outline in U.S. EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, EPA-660/3-75-009; U.S. EPA Acquisition and Culture of Research Fish, Rainbow Trout, Sheepshead Minnows, Channel Catfish and Bluegill Sunfish, EPA-660/3-75-001; U.S. EPA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation, Wildlife and Aquatic Organisms, EPA-540/9-82-024; U.S. EPA Standard Evaluation Procedure, Acute Toxicity Test for Freshwater Fish, EPA-540/9-85-006; and ASTM Standard Guide for Conducting Acute Toxicity Test with Fishes, Macroinvertebrates and Amphibians, ASTM Standard E729. The following guideline deviations were noted:

1. The weight of the control fish at test termination (0.26; 0.17-0.48 g) was lower than recommended (0.5-5.0 g).
2. The age of the test organisms was not reported.
3. The size of the test vessels (22 L) was larger than recommended (18 L).
4. The reported hardness of the dilution water (40-60 mg/L as  $\text{CaCO}_3$ ) exceeded the recommended values (40-48 mg/L as  $\text{CaCO}_3$ ). The reported pH values of the dilution water (7.6-8.1) were exceeded the recommended ranges for estuarine fishes (7.7-8.0).

These deviations did not negatively impact the acceptability of the study.

**COMPLIANCE:** Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with Good Laboratory Practices of the U.S. Environmental Protection Agency, Final Rule (40 CFR, Part 160), Federal Register, Vol 54, No. 158:34067-34074.

**A. MATERIALS:**

**1. Test material** AE 0317309 Technical

**Description:** Light Brown Powder

**Lot No./Batch No. :** Op. 1-4 (Batch No.)

**Purity:** 95.4%

**Stability of compound under test conditions:** Analytical verification of the test material was conducted at 0- and 96-hours. At 0-hours, the recoveries were 97.5-99.7% of nominal; at 96-hours, the recoveries were 101.4-124.7% of nominal. The sample that yielded 124.7% of nominal was reanalyzed in duplicate and yielded recoveries of 100.4 and 98.1% of nominal. Therefore, the 124 mg a.i./L was not used in the mean-measured concentration calculation.

*(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)*

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**Storage conditions of  
test chemicals:**

Stored under ambient laboratory conditions.

**Physicochemical properties of AE 0317309.**

Parameter	Value	Comment
Molecular weight	362.3 g/mol	
Water Solubility (g/L) at 20°C	4.2 at pH 4 69.1 at pH 7 49.0 at pH 9	Very soluble
Vapor Pressure/Volatility	$2.7 \times 10^{-7}$ Pa at 20°C $6.8 \times 10^{-7}$ Pa at 25°C	Non-volatile
UV Absorption	water $\lambda_{\max} = 264$ 0.1M HCl $\lambda_{\max} = 241$ 0.1M NaOH $\lambda_{\max} = 216$	Not likely to undergo photolysis.
Pka	$4.2 \pm 0.15$	
log K <sub>ow</sub> at 23°C	0.276 at pH 4 -1.362 at pH 7 -1.58 at pH 9	Not likely to bioaccumulate
Stability of compound at room temperature, if provided		No significant degradation over 12 months at ambient temperatures.

Data obtained from pyrasulfatole chemistry review of Submission 2006-2445.

**2. Test organism:**

**Species:**

Sheepshead Minnow (*Cyprinodon variegates*) EPA recommends a cold water species (preferably rainbow trout *Oncorhynchus mykiss*) and a warm water species (preferably bluegill sunfish *Lepomis macrochirus*). OECD recommends choice of species at discretion of testing laboratory.

**Age at test initiation:**

Not reported

**Weight at study initiation:**

0.26 (0.17-0.48) g; based on control fish at test termination

EPA recommends: mean 0.5 - 5 g.

**Length at study initiation:**

21.3 (19.0-26.0) mm; based on control fish at test termination

EPA recommends: Longest not > 2x shortest; OECD recommends 2.0  $\forall$  1.0 cm for bluegill and 5.0  $\forall$  1.0 cm for rainbow trout

**Source:**

Aquatic Biosystems, Inc., Fort Collins, Colorado

EPA recommends that all organisms be from the same source

**B. STUDY DESIGN:**

**1. Experimental Conditions**

a. Range-finding study: The study authors reported that the definitive toxicity test was conducted as a limit test because no toxicity was expected based the results of a preliminary range-finding test. The actual results of the range-finding test were not reported.

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b. Definitive Study

**Table 1: Experimental Parameters**

Parameter	Details	Remarks
		Criteria
<u>Acclimation</u>		
Period:	At least 14 days	The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days. Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; $\geq 5$ and $\leq 10\%$ = continued acclimation for 7 days; <5% = acceptable.
Conditions: (same as test or not)	Same as definitive test	
Feeding:	Fish were fed newly hatched brine shrimp nauplii and/or a commercial fish food (Tetramin™, Trout Chow) daily. Fish were not fed 48-hours prior to, and during, the definitive test.	
Health: (any mortality observed)	All fish appeared healthy.	
Duration of the test	96-hours	The recommended test duration is 96 hours.
<u>Test condition</u>		
Static/flow-through	Static	A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.
Type of dilution system - for flow-through method.	N/A	
Renewal rate for static renewal	N/A	
Aeration, if any	Aeration was not provided during the definitive test	Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.

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Parameter	Details	Remarks
		<i>Criteria</i>
<u>Test vessel</u>		
Material: (glass/stainless steel)	Glass	The size of the test vessels (22 L) was larger than recommended (18 L). This is acceptable for OECD and OPPTS 850 Guidelines.
Size:	22 L	<i>Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm.</i>
Fill volume:	18 L	<i>Fill volume is usually 15-30 L of solution.</i>
Source of dilution water Quality:	The dilution water was a mixture of spring water blended with reverse osmosis water to produce soft (40-60 mg/L) water. The spring water was collected from a spring box, passed through a multimedia filter, a 5 micron bag filter, granular activated carbon filters, a 1 micron cartridge filter and finally an ultraviolet sterilizer. The water was then blended with dechlorinated city water which had also been filtered and softened. Artificial sea salts were added to the dilution water to produce a salinity of approximately 15 parts per thousand.	<i>Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water</i> <i>(<a href="http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf">http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf</a>)</i> <i>Dilution water should be intensely aerated before the study. OECD permits dechlorinated tap water.</i>

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Parameter	Details	Remarks
		Criteria
<u>Water parameters:</u>		
Hardness	40-60 mg/L as CaCO <sub>3</sub>	The reported hardness of the dilution water (40-60 mg/L as CaCO <sub>3</sub> ) exceeded the recommended values (40-48 mg/L as CaCO <sub>3</sub> ). The reported pH values of the dilution water (7.6-8.1) were exceeded the recommended ranges for estuarine fishes (7.7-8.0). The pH and hardness are acceptable for OECD and OPPTS 850 Guidelines.
pH	7.6-8.1	
Dissolved oxygen	5.6-7.6 mg/L (71-92% DO saturation)	
Total Organic carbon	0.98 mg/L (spring water) 2.8 (RO water)	
Particulate Matter	4 mg/L	
Metals	Arsenic (0.013 mg/L), Boron (0.220 mg/L, Calcium (120 mg/L), Magnesium (12 mg/L), Manganese (0.0084 mg/L), Potassium (2 mg/L), and Sodium (44 mg/L) were detected in the Spring water; Boron (0.150 mg/L), Calcium (8.9 mg/L), Chromium (0.0055 mg/L), Lead (0.033 mg/L), Magnesium (0.690 mg/L) and Sodium (3.6 mg/L) were detected in the Reverse Osmosis water.	<u>Hardness:</u> EPA recommends 40 - 48 mg/L as CaCO <sub>3</sub> (OECD recommends 10 - 250 mg/L) <u>pH:</u> EPA recommends 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8; (OECD recommends pH 6.0 - 8.5) <u>Dissolved Oxygen:</u> EPA recommends: Static: ≥ 60% during first 48 hrs and ≥ 40% during second 48 hrs; flow-through: ≥ 60%; (OECD guideline recommends at least 80% saturation value). <u>Temperature:</u> EPA recommends 12 EC for coldwater species, 17 or 22 EC for warmwater species, and 22 ± 1 EC for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout). <u>Salinity:</u> EPA recommends 30-34‰ (parts per thousand) for marine, 10-17‰ for estuarine fish, weekly range < 6‰  Water quality should be measured at beginning of test and every 48 hours.
Pesticides	None detected	
Chlorine	<0.004 mg/L	
Temperature	21.2-22.8°C	
{Salinity for marine or estuarine species}	17 ppt	
Intervals of water quality measurement	Temperature, pH, DO and salinity were measured in each replicate test vessel at 0-, 24-, 48-, 72- and 96-hours. Temperature was also measured hourly in an aquarium centrally located in the water bath.	
<u>Number of replicates/groups:</u>		A solvent control was not used.

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Parameter	Details	Remarks
		Criteria
		<i>Recommended number of replicates include a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.</i>
<u>Number of organisms per replicate /groups:</u> control: solvent control: treated ones:	10 N/A 10	One of the control replicates accidentally received 12 fish instead of 10, for a total of 32 control fish.  <i>Number of organisms per replicate should be <math>\geq 10</math>/concentration; OECD guideline recommends at least 7 fish/concentration.</i>
Biomass loading rate	0.17 g/L	<i>Recommended static conditions are #0.8 g/L at #17EC and #0.5 g/L at &gt; 17EC. Recommended flow-through conditions are #1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.</i>
<u>Test concentrations:</u> nominal:  measured:	0 (negative control) and 100 mg a.i./L  <9.92 (<LOQ; negative control) and 100 mg a.i./L	Measured concentrations in 100 mg a.i./L treatments on Days 0 and 96 ranged from 97.5 to 104.4 mg a.i./L, with a mean of 100.2 mg a.i./L.
Solvent (type, percentage, if used)	N/A; a solvent control was not used	<i>The solvent should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg/L.</i>
Lighting	16h light and 8h dark, with a 30 minute transition period to simulate dawn and dusk	<i>The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.</i>
Feeding	Fish were not fed during the definitive toxicity test.	<i>Fish should not feed during the study.</i>



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Parameter	Details	Remarks
		Criteria
<u>Recovery of chemical</u> Frequency of determination Level of quantation Level of detection	0- and 96-hours 9.92 mg a.i./L Not reported	
Positive control {if used, indicate the chemical and concentrations}	N/A; a positive control was not used	
Other parameters, if any	None	

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**2. Observations:**

**Table 2: Observations**

Parameter	Details	Remarks
		Criteria
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	0-, 24-, 48-, 72- and 96-hours	Observation intervals should be a minimum of every 24 hours.
Were raw data included?	Yes	
Other observations, if any	None	

**II. RESULTS AND DISCUSSION:**

**A. MORTALITY:**

By test termination, no mortality was observed in the negative control or in the mean-measured 100 mg a.i./L treatment level. Based on mortality, the LC<sub>50</sub> and NOAEC values were >100 and 100 mg a.i./L, respectively.

**Table 3: Effect of AE 0317309 on Mortality of *Cyprinodon virgatus*.**

Treatment (mg a.i./L) Mean-Measured (and Nominal)	No. of fish at start of study	Observation period					
		Day 1		Day 2		Day 4	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
<9.92 (Negative Control)	32	0	0	0	0	0	0
100 (100)	30	0	0	0	0	0	0
NOAEC	100 mg a.i./L						
LC <sub>50</sub>	>100 mg a.i./L						
Positive control, if used mortality: LC <sub>50</sub> :	N/A; a positive control was not used						

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**B. NON-LETHAL TOXICITY ENDPOINTS:**

By test termination, no sub-lethal effects were observed in the negative control or in the mean-measured 100 mg a.i./L treatment level. The EC<sub>50</sub> and NOAEC values based on sub-lethal effects were >100 and 100 mg a.i./L, respectively.

**Table 4: Sub-lethal Effect of AE 0317309 on *Cyprinodon virgatus*.**

Treatment (mg a.i./L) Mean-Measured (and Nominal)	Observation period		
	endpoint 1 at Day 1	Endpoint 2 at Day 2	endpoint 3 at Day 4
	% affected	% affected	% affected
<9.92 (Negative Control)	All Normal	All Normal	All Normal
100 (100)	All Normal	All Normal	All Normal
NOAEC	100 mg a.i./L		
LOAEC	>100 mg a.i./L		
EC <sub>50</sub>	>100 mg a.i./L		
Positive control, if used % sublethal effect: EC <sub>50</sub> :	N/A; a positive control		

**C. REPORTED STATISTICS:**

No mortality occurred during the exposure period; therefore no statistical procedures were necessary. The LC<sub>50</sub> was described as greater than the highest concentration tested.

**D. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method(s): Due to the complete lack of mortality and sub-lethal effects observed in the negative control and mean-measured 100 mg a.i./L treatment level, all toxicity values were visually determined based on the mean-measured treatment concentration.

LC<sub>50</sub>: >100 mg a.i./L      95% C.I.: N/A

NOAEC: 100 mg a.i./L

Probit Slope: N/A      95% C.I.: N/A

**E. STUDY DEFICIENCIES:**

There were no study deficiencies.

**F. REVIEWERS' COMMENTS:**

The reviewers' results were identical to those of the study author's.

The analysis of the nominal 100 mg a.i./L treatment level at 96-hours yielded a measured concentration of 124.7 mg a.i./L. This treatment level was reanalyzed in duplicate and yielded measured concentrations of 100.4 and 98.1 mg

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a.i./L. Since the Day 0 measurement was 99.7 mg a.i./L and the reanalysis measurements were 100.4 and 98.1 mg a.i./L, the 124.7 mg a.i./L measurement was considered to be a sample analysis error, and, therefore, was not included in the calculation of the 96-hour mean-measured concentration.

The periodic screening analysis of the spring and RO water indicated the presence of metals (arsenic, boron, calcium, chromium, lead, magnesium, manganese, potassium, and sodium); however, no mortality occurred and all fish appeared healthy during the acclimation period and during the 96-hour definitive toxicity test. Therefore, the reviewers did not feel that the presence of these metals had a deleterious affect on the acceptability of the study.

The in-life portion of the definitive acute toxicity test was conducted between January 26 and January 30, 2004.

**G. CONCLUSIONS:**

This study is scientifically sound and is classified as **ACCEPTABLE**. Due to the complete lack of mortality and sub-lethal effects, the LC<sub>50</sub>, EC<sub>50</sub> and NOAEC values were >100, >100 and 100 mg a.i./L, respectively.

**III. REFERENCES:**

- American Public Health Association. 1998. **Standard Methods for the Examination of Water and Wastewater**; 17<sup>th</sup> Edition. Washington, D.C.
- American Society for Testing and Materials (ASTM). 1996. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians. ASTM Standard E729. Philadelphia, PA.
- SAS Institute. 1999. PC-SAS version 8.2. Cary, NC.
- Schneider, J. 2001. Physical and Chemical Properties of JAU 6476. Bayer AG, Leverkusen, Germany. Laboratory Project ID: 14 0120 0950.
- Stephan, C.E. 1977. Methods for Calculating an LC50. In: American Society for Testing and Materials. **Aquatic Toxicology and Hazard Evaluation**, F.L. Mayer and J.L. Hamelink, Eds. ASTM STP 634. Philadelphia, PA. pp 65-84.
- Stephan, C.E. *et al.* 1984. TOXCALC- PC based program for calculating LC50.
- USEPA. 1975a. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. EPA-660/3-75-009. Office of Research and Development, Corvallis, OR. 61 pp.
- USEPA. 1975b. Acquisition and Culture of Research Fish: Rainbow Trout, Sheepshead Minnow, Channel Catfish and Bluegill Sunfish. EPA-660/3-75-011. Office of Research and Development, Corvallis, OR. 45 pp.
- USEPA. 1982. Pesticide Assessment Guidelines, Subdivision E- Hazard Evaluation: Wildlife and Aquatic Organisms. EPA-540/9-82-024. Office of Pesticide Programs, Washington, D.C. 86 pp.
- USEPA. 1985. Standard Evaluation Procedure, Acute Toxicity Test for Freshwater Fish. EPA-540/6-85-006. Office of Pesticide Programs, Washington, D.C.
- USEPA. 1989. Pesticide Programs; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). Federal Register, Vol. 54, No. 158:34067-34074.