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Shaughnessy No.

Data Evaluation Record

# PHMB (Vantocil IB)

Bluegill Sunfish Flow-through Acute Toxicity Test

## 1. TEST MATERIAL: Vantocil IB, PHMB

## 2. STUDY MATERIAL -

| | |
|---|---|
| Poly(hexamethylenebiguanide)hydrochloride | 20% w/w |
| Inert ingredients (unspecified) | 80 |

## 3. STUDY TYPE:

Freshwater Fish Acute Toxicity, Flow-through.

Species tested- Bluegill sunfish *Lepomis macrochirus*

## 4. STUDY IDENTIFICATION:

Roberts, C.G., J.E. Caunter, and J.M. Shearing. 1993. Vantocil IB: Acute toxicity to Bluegill sunfish (*Lepomis macrochirus*). Brixham Environmental Laboratory, Zeneca Limited, Brixham Devon TQ5 8BA, UK. Study number X022/B. Sample number X022. Submitted by Zeneca Specialties, P.O. Box 751, Wilmington, DE 19897. MRID 427221-01. D190392. S439050. Case No. 816384.

## 5. REVIEWED BY:

James J. Goodyear, Ph.D. Signature: James Goodyear
Biologist, Section 1
Ecological Effects Branch Date: 5-17-95
Environmental Fate and Effects Division (7507C)

## 6. APPROVED BY:

Leslie W. Touart, Ph.D. Signature: L W Touart
Head, Section 1
Ecological Effects Branch Date: 5/23/95
Environmental Fate and Effects Division (7507C)

## 7. CONCLUSIONS: The study is invalid because the test solutions were not measured.

## 8. COMMENTS - N/A.

## 9. BACKGROUND: Phase IV study.

INVALID

# 10. DISCUSSION OF INDIVIDUAL TEST - N/A.

# 11. MATERIALS AND METHODS:

## A. TEST CONDITIONS:

*Animals* - Bluegill sunfish (*Lepomis macrochirus*) from Sea Plantations, inc., 27 Congress Street, Salem, Massachusetts. Weight- 0.52 to 2.07 g, mean = 1.18 g. Length- 31 to 45 mm, mean = 38 mm.

*Containers* - Glass vessels (610 X 305 X 310 mm) 54 liters with 45 liters of liquid.

*Loading rate*- "In the dilution control water was 0.52 g/l."

*Solution* - Renewed at 250 ml/minute, 95% exchange in 9 hours. Tap water dechlorinated with sodium thiosulfate. The water was charcoal filtered to remove particle, ultraviolet sterilized and passed through filters with 25 and 10 $\mu$ pores.

"Samples of the stock solution were taken for analysis at 0, 48, and 96 hours."

*Temperature* - 22.0 $\pm$1.0°C.

*Duration* - 96 hours.

*pH* - 7.16 to 7.72.

*Dissolved* $O^2$ - 8.4 to 8.8 mg/l.

*Hardness* - 34.0 to 42.3 mg/l as $CaCO_3$

*Photoperiod* - 16 hours light and 8 hours of darkness with a ten minute transition twice a day.

## B. Dose:

"Dilution water control and nominal concentrations of 0.18, 0.32, 0.56, 1.0, 1.8, and 3.2 mg/l," [page 7]. Table one [page 14] lists "Nominal concentrations of Vantocil IB mg/l- 36, 64, 112, 200, 360, 640" for the stock solutions.

Measured concentrations- Table 3 [page 16] list the "calculated concentrations" of 0.18, 0.32, 0.56, 1.0, 1.8, and 3.2 mg/l. These figures were obtained by dividing the stock concentrations by 200 to account for dilution at 1:200.

"Since it was not possible to measure the level of Vantocil IB in the test solutions, Vantocil IB was measured in the stock solutions. The flow rate for the dilution water and each Vantocil IB stock solution were measured to ensure correct dosing of the test material. The concentration of Vantocil Ib in the test solutions was calculated by dividing the measured concentration in the stock solution by the dilution ratio (200) [page 11]." "Stock solutions were replaced at 48 h. Data for 0 hr and 48 hr show the initial concentrations of Vantocil IB is the stock containers. The 96 h analysis was of the stocks introduced at 48 h to show no loss of the test material between stock renewals [page 14]."

## C. DESIGN:

Twenty fish were randomly assigned to each test concentration and to the control. A continuous flowthrough system was used to feed stock solution to the test vessels. The stock solution was 200 time the desired final concentration. The stock solutions were sampled and analyzed at 0, 48, and 96 hours. Temperature, dissolved oxygen, and pH were measured every day.

## D. STATISTICS- Stephan, *et al.*, 1977.

# 12. REPORTED RESULTS:

The 96-hour flow-through toxicity of Vantocil IB to Bluegill sunfish (*Lepomis macrochirus*) is:

$LC_{50}$ 0.57 mg/l C.I. = 0.46 to 0.70 mg/l NOEC = 0.17 mg/l
Moving average method.

# 13. STUDY AUTHORS' CONCLUSIONS/QA MEASURES:

"This study has been conducted in compliance with the principles of Good Laboratory Practice (GLP) laid down in the United Kingdom Department of Health Compliance Programme (1989)" * * *

"The conduct of this study has been inspected/audited in accordance with Zeneca Limited's policies and procedures for Good Laboratory Practice, . . . The methods and results incorporated in the final report accurately reflect the raw data produced during the study."

# 14. REVIEWER'S DISCUSSION AND CONCLUSIONS:

## A. TEST PROCEDURES:

EEEB's SEP (Zucker, 1985) does not recommend the use of dechlorinated tap water, but the practice is not forbidden. The use of 20 fish per level compensated for the single death in the control tank and is good practice. The rate of flow of solution into the tanks was "95% exchange in 9 hours." This is approximately equal to 2.5 changes per 24 hour period. The SEP recommends five to ten volumes per 24 hours.

## B. STATISTICAL ANALYSIS:

Stephan, C.E. *et al.*, 1978.

$LC_{50}$ 0.59 mg/l C.I. = 0.33 to 0.98 mg/l Binomial test

"When there are less than two concentrations at which the percent dead is between 0 and 100, neither the moving average nor the probit method can give any statistically sound results [computer printout]."

## C. DISCUSSION/RESULTS:

The laboratory did not analyze the test concentrations because "it was not possible. They do not state why it wasn't possible. They said that there was no significant change in the concentration of the stock solutions between the 48 hour and the 96 hour analysis. Testing of test concentrations is necessary because the concentrations in the test tanks might have been effected by the biological effects of 20 fish living in it for 96 hours. The final concentrations might have been different from the calculated levels because of a faulty diluter. They also may have been changed by the degradation of the chemicals because of different pHs, concentrations, movement of the water, *etc.* Testing of the test solutions is standard laboratory practice and would only be waived under exceptional circumstances.

## D. ADEQUACY OF THE STUDY:

*Classification* - Invalid.

*Rationale* - The test concentrations were not measured.

*Repair* - None.

## 15. COMPLETION OF ONE-LINER FOR STUDY:

No

## 16. CBI APPENDIX - N/A.

## LITERATURE CITED

Stephan, C.E. 1977. Methods for calculating an $LC_{50}$. *in*, Aquatic toxicology and hazard evaluation. ASTM STP 634. F.L. Mayer and J.L. Hamelink, Eds. American Society for Testing and Materials. pp. 65-84.

Stephan, C.E., K.A. Busch, R. Smith, J. Burke and R.W. Andrews. 1978. A computer program for calculating an $LC_{50}$. U.S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August, 1978.

Zucker, Elizabeth. 1985. Standard Evaluation Procedure: Acute Toxicity Test for Freshwater Fish. USEPA, Office of Pesticide Programs, Washington, DC. EPA-540/9-85-006.