# Data Evaluation Report on the Acute Toxicity of Fipronil Metabolite RPA 200766 to Freshwater Invertebrates - *Chironomus riparius*

PMRA Submission Number{.....} EPA MRID Number 463767-01

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| **Data Requirement:** | PMRA Data Code | {.....} |
| | EPA DP Barcode | 301708 |
| | OECD Data Point | 202 |
| | EPA MRID | 463676-01 |
| | EPA Guideline | 72-2 |

**Test material:** RPA 200766 (a metabolite of fipronil) **Purity:** 99.8 %
Common name: Reg. No. 5033605 (metabolite of BAS 350 I)
Chemical name: IUPAC: Not reported
CAS name: 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carboxamide
CAS No.: Not available
Synonyms

**Primary Reviewer:** William Evans, Biologist, EPA/OPP/EFED/ERB 1 **Date:** 1/31/05
**{EPA/OECD/PMRA}**

William Evans 2/8/05

**Secondary Reviewer(s):**{.....} **Date:** {.....} **{EPA/OECD/PMRA}**

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

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| **Company Code** | {.....} | [For PMRA] |
| **Active Code** | {.....} | [For PMRA] |
| **EPA PC Code** | 129121 | |

**Date Evaluation Completed:** 1/31/05

**CITATION:** Funk, M, et.al., 2004, Effect of Reg. No. 5033605 (Metabolite of BAS I, RPA 200766 on the Mortality of *Chironomus riparius* in a 48 hour Static, Acute Toxicity Test. BASF Agricultural Center Limbugerhof, Crop Protection Division, Ecology and Environmental Analytics, P.O. 120, 67114 Limburgerhof, Germany.

**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 freshwater invertebrates. 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 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:**

The 48-hour acute toxicity of RPA 200766 (a metabolite of fipronil) to *Chironomus riparius* (a sediment dwelling aquatic invertebrate was studied under static conditions. Larva were exposed to control, solvent control, and test chemical (measured) at 0.008, 0.026, 0.09, 0.26, 0.87, and 2.56 mg/L} for 48 hr. Mortality and sublethal effects were observed daily. The 48-hour $LC_{50}$ was 0.43 mg /L. The 48-hr NOEC based on sublethal adverse effects was {0.008 mg/L}. The sub-lethal effects included were partial paralysis.

Based on the results of this study, RPA 200766 (a metaboltie of fipronil) would be classified as highly toxic to *Chironomus riparius*, a sediment dwelling aquatic invertebrate in accordance with the classification system of the U. S. EPA.

This study is scientifically sound, but does not satisfy US EPA guideline requirements for an acute toxicity study with freshwater invertebrates. It is classified as Supplemental.

**Results Synopsis**

Test Organism Age: < 3 days old at test initiation
Test Type: Static

$LC_{50}$: {0.43 mg/L} 95% C.I.: {0.25 to 0.82 mg/L}
NOEL:{0.008 mg/L} Probit Slope: {1.17}
LOEL: {0.026....mg a.i./L} Endpoint(s) Affected: immobility (partial paralysis)

## I. MATERIALS AND METHODS

**GUIDELINE FOLLOWED:** OECD guideline for testing of chemicals No. 202 "Daphnia Acute Immobilization Test" (1984); the U.S. EPA OPPTS No. 850.1010 (Draft, 1996); and ASTM Standard E729-88a (1994). Deviations from U.S. EPA §72-2 included:

1. The stability of the compound was not reported. OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound.

2. Continuous laboratory cultures were maintained for 3 days. EPA recommends a minimum of 7 days.

3. The test vessel size was not reported and fill volume was 30 mL. EPA static test are usually conducted in 250 mL beakers with 200 mL of solution.

4. Treatment concentrations should include a geometric series of at least five concentrations plus a control with each recommended concentration being at least 60% of the next higher one. Concentrations were about 30% of the next higher one.

5. Level of Quantitation and Level of Detection should have been reported.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in accordance with GLP standards of OECD (ENV/MC/CHEM (98)17) and the GLP Principles of the German "Chemikaliengesetz" (Chemicals Act).

**A. MATERIALS:**

**1. Test material** Reg. No. 5033605 Metabolite of BAS I, RPA 200766

**Description:** Soluble white powder

**Lot No./Batch No. :** 73PAC1

**Purity:** 99.8%

**Stability of compound under test conditions:** Not reported. However, acetone was used as a solvent. *(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)*

**Storage conditions of test chemicals:** Cool and dry at 5°C. Allowed to reach ambient temperature before opening to avoid problems with condensation.

**2. Test organism**:

**Species:** *Chironomus riparious* (EPA preferred species is *Daphnia magna*)
**Age at test initiation**: < 3 days old
**Source:** In house cultures

**B. STUDY DESIGN:**

**1. Experimental Conditions**

a. Range-finding study : No range-finding study was conducted.

b. Definitive Study

**Table X . Experimental Parameters**

| Parameter | Details | Remarks |
|---|---|---|
| Acclimation period:<br><br>Conditions: (same as test or not)<br>Feeding:<br><br>Health: (any mortality observed) | Test initiation: 4/30/04.<br>Experimental start date: 5/3/04 (72 hours)<br>Same as test<br>Not mentioned, but presumed not to be fed.<br>No mortalities were observed in any control | *The recommended acclimation period is a minimum of 7 days.*<br>*Organisms should not feed during the study* |
| Duration of the test | 48 hours | *The recommended test duration is 48 hours* |
| Test condition static/flow-through<br><br>Type of dilution system (for flow-through method).<br><br>Renewal rate for static renewal | Static<br><br>N/A<br><br>N/A | *The recommended flow rates are 5 - 10 volume additions/24 hours; meter systems should be calibrated before the study and checked twice daily during the test period* |
| Aeration, if any | Aerated to saturation | |

| Parameter | Details | Remarks |
|---|---|---|
| Test vessel<br><br>Material: *(glass/stainless steel)*<br>Size:<br>Fill volume: | <br><br>Glass beakers<br>50 mL<br>30 mL | <br><br>*Flow-through studies are usually conducted in 3.9 L or 1 gallon jars with 2-3 l of solution; static tests are usually conducted in 250 ml beakers with 200 ml of solution.* |
| Source of dilution water | Reconstituted water, M4 according to Elendt prepared on the basis of an ultrapure deionized water. | *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 (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010pdf). Dilution water should be intensely aerated before the study.* |
| Water parameters:<br><br>Hardness<br>pH<br>Dissolved oxygen<br>Temperature<br>Total Organic Carbon<br>Particulate matter<br>Metals<br>Pesticides<br>Chlorine | <br><br>2.55 mmol/L<br>7.97<br>8.6 - 8.73 mg/L at test initiation<br>21 - 21.5°C<br>Not reported<br>Not reported<br>Not reported<br>Not reported<br>Not reported | <br><br>*Recommended hardness is 40-48 mg/L of $CaCO_3$. (OECD recommends <250 - >140 mg/L.*<br>*Recommended pH is 7.2 - 7.6. (OECD recommends pH of 6-9).*<br>*Recommended temperature is 20°C (measured continuously or if water baths are used, every 6 hr; temperature should not vary > 1°C. (OECD recommends 18°-22°C within ± 1°C ).*<br>*Dissolved oxygen: recommended flow-through or static conditions are ≥ 60% during test duration.*<br>*Water quality should be measured at beginning of test and every 48 hours.* |

| Parameter | Details | Remarks |
|---|---|---|
| Number of replicates<br>Solvent control:<br>Treatments: | <br>4<br>4 | |
| Number of organisms per replicate<br>Solvent control:<br>Treatments: | 5 larva per replicate for all controls | *At least 20 organisms should be exposed at each treatment level. Recommended biomass loading rate for static condition is ≤ 0.8 g/L at ≤ 17°C and ≤ 0.5 g/L at > 17°C. Recommended flow-through condition is ≤ 1 g/L/day.* |
| Treatment concentrations<br>nominal:<br><br>measured: | 0(negative and solvent controls), 0.1, 0.33, 0.1, 0.33, 1.0, and 3.0 ppm<br><br>(LOQ not reported for controls) 0.008, 0.026, 0.09, 0.26, 0.87, and 2.56 ppm | *Treatment concentrations should include a geometric series of at least five concentrations plus a control with each recommended concentration being at least 60% of the next higher one.* |
| Solvent (type, percentage, if used) | Acetone; 240 µL/100 mL (0.00024 mL/L) | *Solvents should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-though tests.* |
| Lighting | 16 hours light/8 hours dark and 130 - 200 lux | *Recommended photoperiod is 16 hours of light and 8 hours of dark.* |
| Stability of chemical in the test system | Verified. Recoveries averaged 82.1% of nominal at test initiation and 83.3% at test termination | |
| Recovery of chemical<br><br>Level of Quantitation<br>Level of Detection | 75.8 - 89.8% of nominal<br><br>Not reported<br>Not reported | |
| Positive control {if used, indicate the chemical and concentrations} | N/A | |
| Other parameters, if any | N/A | |

**2. Observations:**

**Table X: Observations**

| Criteria | Details | Remarks |
|---|---|---|
| Parameters measured including the sublethal effects | Mortality/immobility and sub-lethal effects (partial paralysis) | |
| Observation intervals | 24 and 48 hours | |
| Were raw data included? | Yes | |
| Other observations, if any | N/A | |

## II. RESULTS AND DISCUSSION

### A. MORTALITY:

Mortality was observed at the test concentration level of 0.09 mg/L and higher after 24 and 48 hours. There were no mortalities in the control. (*EPA's Standard Evaluation Procedure (SEP) includes guidance that pretest mortality should be ≤ 3% 48 hours prior to testing and control mortality should be ≤ 10% at end of study*)

**Table 3: Effect of RPA 200766 on Mortality of *Chironomus raparius***

| Treatment (mg a.i./L) Measured and (nominal) conc. | No. of organisms | Observation period | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2 Hours | | 24 Hours | | 48 Hours | |
| | | No Dead | % mortality | No Dead | % mortality | No Dead | % mortality |
| Solvent Control | 20 | N/A | N/A | 0 | 0 | 0 | 0 |
| Negative Control | 20 | N/A | N/A | 0 | 0 | 0 | 0 |
| 0.01 (0.008) | 20 | N/A | N/A | 0 | 0 | 0 | 0 |
| 0.033 (0.026) | 20 | N/A | N/A | 0 | 0 | 0 | 0 |
| 0.1 (0.09) | 20 | N/A | N/A | 1 | 5 | 5 | 25 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0.33 (0.26) | 20 | N/A | N/A | 1 | 5 | 12 | 60 |
| 1.0 (0.87) | 20 | N/A | N/A | 1 | 5 | 13 | 65 |
| 3.0 (2.56) | 20 | N/A | N/A | 0 | 0 | 14 | 70 |
| NOEC mg ai/L | | N/A | | Not reported | | 0.026 | |
| $LC_{50}$ mg ai/L | | N/A | | Not reported | | 0.25 | |

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

Authors observed sub-lethal behaviors such as partial paralysis at the 0.026 mg/L concentration after 24 hours and these effects continued to increase at the other levels through-out the experiment.

**Table 4: Effect of RPA 200766 on Partial Paralysis- *Chironomus raparius***

| Treatment (mg a.i./L) Measured and (nominal) concentrations | Observation period | | | | | |
|---|---|---|---|---|---|---|
| | 2 Hours | | 24 Hours | | 48 Hours | |
| | end-point | % affected | end-point | % affected | end-point | % affected |
| Solvent Control | N/A | N/A | partial paralysis | 0 | partial paralysis | 0 |
| Negative Control | N/A | N/A | partial paralysis | 0 | partial paralysis | 0 |
| 0.01 (0.008) | N/A | N/A | partial paralysis | 0 | partial paralysis | 0 |
| 0.033 (0.026) | N/A | N/A | partial paralysis | 45 | partial paralysis | 65 |
| 0.1 (0.09) | N/A | N/A | partial paralysis | 80 | partial paralysis | 75 |
| 0.33 (0.26) | N/A | N/A | partial paralysis | 95 | partial paralysis | 40 |
| 1.0 (0.87) | N/A | N/A | partial paralysis | 95 | partial paralysis | 35 |
| 3.0 (2.56) | N/A | N/A | partial paralysis | 100 | partial paralysis | 30 |
| NOEC mg/L | Not reported | | Not reported | | Not reported | |
| $EC_{50}$ mg/L | Not reported | | Not reported | | Not reported | |

**C. REPORTED STATISTICS:** Authors derived the NOEC, $LC_0$, and $LC_{100}$ directly from the data. Determination of the $LC_{50}$ was done by Trimed Spearman-Karbar (TOXSTAT3.5)

**D. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: The 48-hour $LC_{50}/EC_{50}$ and 95% confidence interval were determined using the probit method via TOXANAL software. The NOEC was visually determined as the highest concentration which exhibited no significant mortality/immobility and sub-lethal effects. All toxicity values were determined in terms of the reported mean-measured concentrations.

$LC_{50}$: 0.43 mg/L 95% C.I.: 0.25 - 0.81 mg/L
NOEC: 0.008 mg/L (based on sub-lethal effects)
LOEC: 0.026 mg/L (based on sub-lethal effects)
Probit Slope: 1.18 95% C.I.: 0.79 - 1.56 mg/L
Endpoints affected: Mortality and sub-lethal effects (same conclusions)

**E. STUDY DEFICIENCIES:** As noted in the Materials and Methods section, this study followed the OECD guidelines for testing. However, the study deficiencies did not affect the scientific validity of the study, and therefore, this study is classified SUPPLEMENTAL because it provides useful information on the acute toxicity of RPA 200766 (a metabolite of fipronil) to a sediment dwelling aquatic invertebrate (*Chironomus riparius*).

**F. REVIEWER'S COMMENTS:** The reviewer's conclusions differed from those reported by the study author because the study author based estimated $EC_{50}$ and NOEC values on a different statistical program than the study author. Even though this study is scientifically sound it did not follow the current U.S. EPA FIFRA guidelines §72-2.

**G. CONCLUSIONS:** The study is scientifically sound; however, it was not designed to fulfill the current U.S. EPA FIFRA guideline §72-2. This study is therefore classified SUPPLEMENTAL, as it provides useful information on the acute toxicity of RPA 200766 (a metabolite of fipronil) to a sediment dwelling aquatic invertebrate (*Chironomus riparius*). The $EC_{50}/LC_{50}$ of 0.43 mg/L based on mortality classifies RPA 200766 as highly toxic to aquatic invertebrates. The NOEC and LOEC based on sub-lethal effects is 0.008 and 0.026 mg/L, respectively.

$EC_{50}$: 0.43 mg/L 95% C.I.: 0.25 - 0.81 mg/L
NOEC: 0.008 mg/L (based on sub-lethal effects)
LOEC: 0.026 mg/L (based on sub-lethal effects)
Probit Slope: 1.18 95% C.I.: 0.79 - 1.56 mg/L
Endpoints affected: Mortality and sub-lethal effects (same conclusions)

**III. REFERENCES:**

Stephan, C.E. 1977. "Methods for Calculating an $LC_{50}$" in: Mayer, F.L. and Hamelink, J.L. (Eds.) *Aquatic Toxicology and Hazard Evaluation*, p. 65-84. ASTM STP 634.

**APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL RESULTS:**

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .1934929

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 4 | .1155016 | .3806707 | .2232099 | .7111323 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 6 | .1064392 | 1 | .1091308 |

SLOPE = 1.17704
95 PERCENT CONFIDENCE LIMITS = .7930301 AND 1.561049

LC50 = .4337278
95 PERCENT CONFIDENCE LIMITS = .2549277 AND .8197598

LC10 = 3.616089E-02
95 PERCENT CONFIDENCE LIMITS = 1.114575E-02 AND .0721085