



Data Evaluation Report on the toxicity of BAS 670 00 H to the parasitic wasp, *Aphidius rhopalosiphi*.

PMRA Submission Number 2003-0839

EPA MRID Number 45901815

**Data Requirement:** PMRA DATA CODE: 9.2.6 (non-target terrestrial invertebrates - parasites)  
EPA DP Barcode: D290076  
OECD Data Point:  
EPA Guideline: OPPTS None; OPP None

**Test material:** BAS 670 00 H **Purity (%):** 351.6 g a.i./L (31% a.i.)

**Common name:** BAS 670 H

**Chemical name:**

**IUPAC:** [3-(4,5-dihydro-isoisoxazol-3-yl)-4-methanesulfonyl-2-methyl-phenyl]-(5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone

**CAS name:** [3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)-

**CAS No.:** 210631-61-8

**Synonyms:** Reg. No. 375080, methanone

**Primary Reviewer (officer number):** 1268  
**PMRA**

**Signature:**

**Date:** September 07, 2004

**Secondary Reviewer:** Stephen Carey, Biologist  
**EPA**

**Signature:** 

**Date:** February 23, 2005

**Company Code:** BAZ  
**Active Code:** MTN  
**Use Site Category:** 14  
**EPA PC Code:** 123009

**CITATION:** Moll, M, and M. Groer. 2001. Effects of BAS 670 00 H on the parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) in the laboratory - dose response design. IBACON, Germany. Study No. 7621001, BASF Corp. Registration No. 2001/1001838. Submitted to PMRA on March 31, 2003.





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**EXECUTIVE SUMMARY:**

This study determined the acute toxicity of BAS 670 00 H (guarantee 351.5 g BAS 670 H/L, equivalent to 31% a.i.) to the parasitic wasp (*Aphidius rhopalosiphi*), and the impact on female reproductive capacity (i.e., parasitism success on aphid hosts). This study was done according to the protocol of Polgar (1988) and was conducted under OECD and Chemikaliengesetz GLP. Dose rates for BAS 670 00 H were 0 (water control), 56, 112, 225, 450, and 675 mL/ha (or 0, 19.7, 39.4, 79.1, 158.2 and 237.3 g a.i./ha), which are up to nine times higher than the recommended label dose rate of 73 mL/ha. Adult wasps were exposed to either water control, test substance, or toxic standard (Perfekthion EC [dimethoate]) for 48 hours. After which, 15 surviving females from the control, 19.7 and 158.2 g a.i./ha treatments were allowed to parasitize host aphids (*Rhopalosiphum padi*) for ~24 hours. Development of aphid mummies was then observed for 11 - 12 days. The following test validity criteria were met: control mortality = 5% (12.5% maximum), mean control reproduction = 11.0 mummies/female, and no parasitoids producing zero values ( $\geq 5$  mummies/female;  $\leq 2$  parasitoids producing zero values), and toxic standard mortality = 100% (minimum of 50%).

Control-corrected mortality in wasps ranged from 0% at 19.7 g a.i./ha to 34.2% at 237.3 g a.i./ha. Mortality in the treatments were significantly different from negative controls at 158.2 and 237.3 g a.i./ha (Fischer exact-test,  $p < 0.05$ ). A 34.5% reduction in parasitization of aphids in wasps exposed to 158.2 g a.i./ha was considered a biologically significant reduction in reproductive capacity. The beneficial capacity of predatory mites (E) was reduced by 22.7 and 50.5% compared to the negative control at concentrations of 19.7 and 158.2 g a.i./ha, respectively. According to the IOBC classification scheme (Hassan 1992), BAS 670 00 H can be classified as Category 2 (slightly harmful) to *Aphidius rhopalosiphi*.

This study is classified as acceptable and satisfies the Canadian conditional guideline requirement for an acute toxicity study with a beneficial terrestrial invertebrate parasite (DACO 9.2.6). This study was designed to fulfill the requirements of the Commission Directive 96/12/EC and/or of the 'SETEC - Guidance Document on Regulatory Testing Procedures for Pesticides with Non-target Arthropods' (Barrett *et al.*, 1994). This study does not fulfill any current U.S. EPA guideline. This study provides useful information on the acute toxicity of the end-use product BAS 670 00 H (31% a.i.) to the parasitoid wasp (*Aphidius rhopalosiphi*).

**Results Synopsis**





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PMRA Submission Number 2003-0839

EPA MRID Number 45901815

LR<sub>50</sub>: >675 mL/ha (>273 g a.i./ha)  
NOER (mortality): 225 mL/ha (79.1 g a.i./ha)  
Endpoints Effected: Mortality, parasitisation rates.

## I. MATERIALS AND METHODS

### GUIDELINE FOLLOWED:

This study was not designed to fulfill any current U.S. EPA guideline. This study was designed to comply with the Guideline for testing the effect of pesticides on *Aphidius matricariae* HAL. (Hymenoptera, Aphidiidae) (Polgar, 1988), with current improvements by the ring-test group. The following deviations from the Polgar guidance occurred, but did not adversely affect the results of the study: a) temperature exceeded  $20 \pm 3^\circ\text{C}$  (to a maximum of  $24^\circ\text{C}$ ) on four days for approximately 3 - 9 h, b) application rate deviation of  $2 \text{ mg/cm}^2 \pm 10\%$  was exceeded (deviation of + 11.1%, or 225 mL product/ha), c) introduction of individuals to occur 1 h after application at the latest; introductions in the 450, and 675 mL product/ha were 1 h 5 min, and 1 h, 10 min, respectively.

### COMPLIANCE:

Study conducted according to GLP: OECD (1997) and Chemikaliengesetz der Bundesrepublik Deutschland (ChemG), Anhang 1 (1994/97). Signed and dated GLP, Quality Assurance and a No Data Confidentiality claim were provided.

### 1. Test Material

BAS 670 00 H

**Description:** grey-beige liquid herbicide  
**Lot No./Batch No. :** 2000-1  
**Purity:** 336 g a.i./L (nominal)

(3)



**Data Evaluation Report on the toxicity of BAS 670 00 H to the parasitic wasp, *Aphidius rhopalosiphi*.**

**PMRA Submission Number 2003-0839**

**EPA MRID Number 45901815**

351.5 g a.i./L (measured; 31% a.i.)

**Stability of Compound  
Under Test Conditions:**

The stability of the test substance under the conditions employed in this study was not determined.

**Storage conditions of  
test chemicals:**

in original container at room temperature (5 - 30°C),  
in the dark

**Density:**

1.134 g/mL

**Table 1. Physicochemical properties of BAS 670 H (active ingredient of BAS 670 00 H).**

Parameter	Values	Comments
Water solubility at 20°C	510 mg/L in deionized H <sub>2</sub> O at 20°C >100 g/L at pH >9	Highly soluble
Vapour pressure	<1.0 x 10 <sup>-12</sup> mbar (= <1.01 x 10 <sup>-10</sup> Pa) at 20°C	Low volatility
UV absorption	207 nm: 0.7637 272 nm: 0.2426 300 nm: 0.1636 410 nm: 0.0027	Potential for phototransformation (i.e. absorbance occurring within 285 - 350 nm range)
pKa	4.06 @ 20°C	Dissociated at environmentally relevant pHs
Log Kow	-1.52 @ 20°C	Not likely to bioaccumulate

**2. Test organism:**

**Species:**

Parasitic wasp (*Aphidius rhopalosiphi*)

**Age at test initiation:**

< 48 h old

**Source:**

PK Nützlingszuchten, Dr. Peter Katz, Industriestr.



**Data Evaluation Report on the toxicity of BAS 670 00 H to the parasitic wasp, *Aphidius rhopalosiphi*.**

**PMRA Submission Number 2003-0839**

**EPA MRID Number 45901815**

**Stage Transported:** 38, D-73642 Welzheim.  
**Cultural Background:** aphid mummies  
None provided.

**B. STUDY DESIGN:**

**1. Experimental Conditions**

**a) Range-finding Study:** Exposure rates in the main test were based on results from the following range-finding test: 0 (control), 7.91, 23.7, 71.1, 213 and 640 mL/ha BAS 670 00 H (or, 2.78, 8.33, 25.0, 74.9 or 225 g a.i./ha).

**b) Definitive Study**

Table 2. Experimental Parameters/Design

<b>Parameter</b>	<b>Value</b>	<b>Remarks Criteria</b>
<u>Acclimation:</u>		O.K.
Duration:	Held under test conditions from delivery of aphids (~ 2 days).	
Feeding:	1:3 solution of honey in water and a solution of 25% fructose on a cotton wool pad	
Health of aphids	Not reported.	



Data Evaluation Report on the toxicity of BAS 670 00 H to the parasitic wasp, *Aphidius rhopalosiphi*.

PMRA Submission Number 2003-0839

EPA MRID Number 45901815

Parameter	Value	Remarks ----- Criteria
Cage - description and size	<p>Hatching chambers: glass tubes 15 cm x 1.5 cm diameter (large end) and 0.5 cm diameter (small end)</p> <p>Exposure units: ventilated aluminum frame with untreated cloth supporting 2 treated glass plates (13 cm x 13 cm)</p> <p>Post-exposure units (parasitisation period): untreated potted barley seedlings infested with host aphids surrounded by a polyacrylic cylinder (30 cm x 10 cm diameter)</p>	<p>O.K.</p> <p>-----</p> <p><i>Polgar (1988), Mead-Briggs (1992): treated glass plates fitted to a square aluminum frame (10 cm x 10 cm) with holes for ventilation.</i></p>
<p><u>Test conditions</u></p> <p>Temperature: acclimatisation: exposure: post-exposure:</p> <p>Humidity: acclimatisation: exposure: post-exposure:</p> <p>Lighting: acclimatisation: exposure: post-exposure:</p> <p>Photoperiod:</p>	<p>Test units held in an environmental chamber</p> <p>19 - 22°C 19 - 23°C 18 - 24°C</p> <p>80 - 85% 80 - 85% 86 - 90%</p> <p>1630 lux 2220 - 2400 lux 2240 - 6430 lux</p> <p>16 h L: 8 h D</p>	<p>O.K.; due to technical reasons, temp. increased to 24°C on four occasions.</p> <p>-----</p> <p><u>Temperature:</u> <i>Polgar (1988): 21°C</i> <i>Mead-Briggs (1992): 16-22°C</i></p> <p><u>Humidity:</u> <i>Polgar (1988): 70-80% RH</i> <i>Mead-Briggs (1992): 50-80% RH</i></p> <p><u>Lighting:</u> <i>Polgar (1988): 16h L: 8h D</i> <i>Mead-Briggs (1992): continuous (~1000 lux)</i></p>
<p><u>Solvent/dispersant control, if used</u></p> <p>Name: Concentration:</p>	<p>none.</p>	<p>-----</p>



**Data Evaluation Report on the toxicity of BAS 670 00 H to the parasitic wasp, *Aphidius rhopalosiphi*.**

**PMRA Submission Number 2003-0839**

**EPA MRID Number 45901815**

Parameter	Value	Remarks Criteria
Number of wasps per test unit Exposure: Post-exposure:	10 per unit (7 f and 3 m) 1 f per unit	O.K. ----- Exposure period: Polgar (1988): 10 f per test unit Mead-Briggs (1992): 5 f and 5 m per test unit Post-exposure period: 1 f per infested plant
<u>Number of replicates per treatment</u> Exposure: Post-exposure:	4 replicates per treatment group 15 replicates (control, 56 mL/ha), 14 replicates (450 mL/ha)	O.K. ----- Exposure period: Polgar (1988): 4 reps Mead-Briggs (1992): 3 reps Post-exposure period: Polgar (1988): 10 f Mead-Briggs (1992): min. 5 f
<u>Doses used</u> Nominal:  Measured:	0 (negative control), 56, 112, 225, 450 and 675 mL/ha  = 0, 19.7, 39.4, 79.1, 158.2 and 237.3 g a.i./ha (based on measured purity)  Not measured.	Proposed field application rate = 23 g a.i./ha
Deposition rate	2 mg/cm <sup>2</sup> (corresponding to 200 L spray liquid/ha)	O.K. lab track sprayer used with TeeJet 8002 EVS at 2.0 bar and 2 km/h spraying speed.  ----- Polgar (1988): 1 mg/cm <sup>2</sup> Mead-Briggs (1992): 2 mg/cm <sup>2</sup>
Duration of the study Exposure: Post-exposure:	24 h parasitisation period: ~ 24 h post-parasitisation period: 11 - 12 d	O.K. -----
Indicate other factors, if any		



**Data Evaluation Report on the toxicity of BAS 670 00 H to the parasitic wasp, *Aphidius rhopalosiphi*.**

**PMRA Submission Number 2003-0839**

**EPA MRID Number 45901815**

Parameter	Value	Remarks ----- Criteria
<u>Reference chemical, if used</u>		O.K.
Name:	Perfekthion EC (400 g/L dimethoate)	
Concentration:	0.3 mL/ha in 200 L water (corresponding to 1.5 µL Perfekthion/L, or 0.06 g/L dimethoate)	

**2. Observations:**

Table 3: Observations

Parameters	Details	Remarks ----- Criteria
Parameters measured including sublethal effects/toxicity symptoms	<p>-Mortality (= number of parasitoids dead or moribund, corrected for mortality in negative controls using Schneider-Orelli's formula)</p> <p>-Reproduction (mean no. of aphid mummies per female within the 24 h parasitisation period) assessed for 2 highest treatments at which up to 30% mortality occurred (in this case, at 56 and 450 mL/ha).</p>	<p>O.K.</p> <p>Mortality in negative control was &lt;12.5%.</p> <p>Mean reproduction rate was <math>\geq 5</math> mummies per female. and <math>\leq 2</math> parasitoids produced zero values.</p> <p>Based on author's criteria, it is not clear why fecundity wasn't assessed for the 225 mL/ha treatment. The 450 level was assessed for reproduction.</p> <p>-----</p> <p><i>Mead-Briggs (1992): max control mortality of 17%; min. 5 females tested for parasitisation rate</i></p>



Data Evaluation Report on the toxicity of BAS 670 00 H to the parasitic wasp, *Aphidius rhopalosiphi*.

PMRA Submission Number 2003-0839

EPA MRID Number 45901815

Parameters	Details	Remarks
		Criteria
Observation intervals	-Mortality: Hours 2, 24 and 48 -Reproduction: Days 11 and 12 after the 24 h parasitisation period	O.K. ----- <i>Polgar (1988), Mead-Briggs (1992):</i> <i>Mortality: Hours 2, 24 and 48</i> <i>Reproduction: 10 - 12 after the 24 h parasitisation period</i>
Were raw data included?	Yes	
Other observations, if any		

**II. RESULTS AND DISCUSSION:**

**A. MORTALITY:**

Mortality in *Aphidius* wasps showed a positive dose-response relationship. Control-corrected mortality after 48 hours increased from 0.0% at 19.7 g a.i./ha, to 34.2% at 237.3 g a.i./ha. Significantly more mortality was found in the two highest concentrations tested (i.e., 158.2 and 237.3 g a.i./ha) compared to controls (Fisher's Exact tests,  $p \leq 0.087$ ). The NOEC is therefore 79.1 g a.i./ha. No LR<sub>50</sub> could be determined as less than 50% mortality was observed at the highest concentration. Control mortality was within the validity criterion (i.e.,  $\leq 12.5\%$ ).

Table 4: Effect of BAS670 00 H on cumulative mortality of parasitic wasps (*A. rhopalosiphi*).

Treatments (g a.i./ha) [nominal conc.]	No. of parasitoids	% Mortality during observation period (Mean $\pm$ S.D.)			
		Hour 2	Hour 24	Hour 48	Corrected Hour 48*
0 (Negative control)	40	0.0 (0.0)	0.0 (0.0)	5.0 (5.8)	-
0.06 g/L dimethoate (Positive control)	40	0.0 (0.0)	97.5 (5.0)	100.0 (0.0)	100
19.7	40	0.0 (0.0)	2.5 (5.0)	5.0 (5.8)	0
39.4	40	0.0 (0.0)	0.0 (0.0)	7.5 (9.6)	2.6
79.1	40	0.0 (0.0)	2.5 (5.0)	12.5 (5.0)	7.9
158.2	40	0.0 (0.0)	0.0 (0.0)	27.5 (18.9)	23.7*



**Data Evaluation Report on the toxicity of BAS 670 00 H to the parasitic wasp, *Aphidius rhopalosiphi*.**

**PMRA Submission Number 2003-0839**

**EPA MRID Number 45901815**

Treatments (g a.i./ha) [nominal conc.]		No. of parasitoids	% Mortality during observation period (Mean ± S.D.)			
			Hour 2	Hour 24	Hour 48	Corrected Hour 48*
237.3		40	0.0 (0.0)	0.0 (0.0)	37.5 (22.2)	34.2*
NOEC		79.1 g a.i./ha				
LR <sub>50</sub>		>237.3 g a.i./ha				
<u>Reference chemical</u>	LC <sub>50</sub>	<0.06 g/L dimethoate				
	NOEC	<0.06 g/L dimethoate				

Note: Mortality data does not include escapees.

\* Day 2 mortality for test substance corrected for negative controls:  $M\% = (t-c)/(100-c) * 100\%$ , where M = % corrected mortality, t = % mortality of test group, and c = % mortality of controls.

\* Significantly different from controls (Fisher's Exact test,  $p < 0.05$ )

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

No sublethal behavioural effects were observed in aphids at all test concentrations. The parasitisation rate was  $11.0 \pm 5.5$ ,  $13.5 \pm 10.0$  and  $7.2 \pm 7.1$  mummies per female in the control, 19.7 and 158.2 g a.i./ha treatments, respectively. The differences between both treatment levels and the control were not significantly different (Dunnnett-test,  $p > 0.05$ ). The percent reduction of reproduction of BAS 670 00 H on *Aphidius rhopalosiphi* was 22.7 and 50.1% at 19.7 and 158.2 g a.i./ha, respectively. Therefore, BAS 670 00 H can be classified as slightly harmful at 158.2 g a.i./ha according to the IOBC classification scheme.

**C. REPORTED STATISTICS:**

Mortality data were analysed for significance using Fisher's Exact test (two-sided,  $p = 0.05$ ). Reproduction data were tested for normality and homogeneity of variance using the Kolmogorov-Smirnov-test ( $p = 0.05$ ) and Cochran-test ( $p = 0.05$ ), respectively, and significant differences were analysed by Dunnnett's test (two-sided,  $p = 0.05$ ).

**D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:**

The mortality data stats were verified using the Fisher's Exact-test and the reproduction data was verified using ANOVA (Dunnnett's test not required). Although the decrease in reproduction rate at 158.2 g a.i./ha was not significantly different from controls (Dunnnett's test,  $p = 0.101$ ), a 34.5% reduction in reproductive capacity relative to controls could represent a significant effect at the population level. Therefore, the LOEC for reproductive capacity should be equal to 158 g





**Data Evaluation Report on the toxicity of BAS 670 00 H to the parasitic wasp, *Aphidius rhopalosiphi*.**

**PMRA Submission Number 2003-0839**

**EPA MRID Number 45901815**

a.i./ha, and the NOEC should be >19.7 and <158.2 g a.i./ha.

**E. STUDY DEFICIENCIES:**

This study was originally rejected in fulfilling PMRA DACO 9.2.6 due to the following deficiency: according to Mead-Briggs (1992), parasitism success should be assessed for each treatment where wasp survival is adequate (recommended minimum of five available females per treatment level for parasitism of aphid larvae). According to this criteria, the authors should have assessed parasitism success for all treatment levels in this study. As a result, a definitive NOEC for reproductive effects could not be established because only two treatment levels were tested (19.7 and 158.2 g a.i./ha). Although this will result in a range for a NOEC, this study was found to conform to the protocol of Polgar (1988), was scientifically sound, and provides adequate mortality and limited reproductive effects data for this parasite.

Although the study was not designed to fulfill any current U.S. EPA FIFRA guideline, it is scientifically valid, and provides useful information on the 48-hour acute toxicity of BAS 670 00 H (containing 31% a.i.) to the predatory wasp, *Aphidius rhopalosiphi*.

**F. REVIEWER'S COMMENTS:**

The study author reported that the application rate was verified by weighing one of the treated glass plates immediately before and after application to determine spray deposit. However, the actual application rates were not provided (only nominal values were reported), and the stability of BAS 670 H (a.i.) was not assessed under actual use conditions during the 48-hour exposure period.

**G. CONCLUSIONS:**

This study is acceptable, and satisfies the Canadian guideline requirement for conditionally required data under DACO 9.2.6. This study was not designed to fulfill any current U.S. EPA FIFRA guideline. The study provides useful information on the acute toxicity of BAS 670 00 H (containing 31% a.i.) to the parasitoid, *Aphidius rhopalosiphi*. Exposure of parasitic wasps (*Aphidius rhopalosiphi*) to BAS 670 00 H resulted in significant mortality at nominal concentrations of 158.2 g a.i./ha and above (NOEC = 79.1 g a.i./ha). Reproductive capacity of parasitic wasps was significantly reduced at 158.2 g a.i./ha. According to the IOBC classification scheme (Hassan 1992), BAS 670 00 H can be classified as Category 2 (slightly harmful) to *Aphidius rhopalosiphi*.



**Data Evaluation Report on the toxicity of BAS 670 00 H to the parasitic wasp, *Aphidius rhopalosiphi*.**

**PMRA Submission Number 2003-0839**

**EPA MRID Number 45901815**

**III. REFERENCES:**

Bakker, F., A. Grove, S. Blümel, J. Calis and P. Oomen. 1992. Side-effect tests for phytoseids and their rearing methods. IOBC/WPRS Bulletin, 15(3): 61-81.

Hassan, S. A. 1992. Meeting of the working group "Pesticides and Beneficial Organisms", University of Southampton, UK, September 1991. IOBC/WPRS Bulletin XV/3: 1-3.

Mead-Briggs, M. A laboratory method for evaluating the side-effects of pesticides on the cereal aphid parasitoid *Aphidius rhopalosiphi* (Destefani-Perez). Aspects of Appl. Biol. 31: 179-189.

Polgar, L. 1988. Guideline for testing the effect of pesticides on *Aphidius matricariae* HAL. (Hymenoptera, Aphidiidae). IOBC/WPRS Bulletin XI/4: 29-34.

Approved 04/01/01 C.K.

12