M-457055-01-3

Dossier According to Directive 91/414/EEC

TERPENOID BLEND (A-TERPINENE, P-CYMENE, D-LIMONENE) QRD 460

Active substance for insect pest control developed from plant extract of *Chenopodium ambrosioides* near *ambrosioides*

DOCUMENT MII, Section 6

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

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8 ECOTOXICOLOGICAL STUDIES OF THE ACTIVE SUBSTANCE

Terpenoid Blend (α-terpinene, p-cymene, and d-limonene) QRD 460 is a new active substance developed by AgraQuest Inc. based originally on the naturally occurring extract of the plant species *Chenopodium ambrosioides* near *ambrosioides* for use as an insecticide plant protection product.

To defend themselves against herbivores and pathogens, plants naturally release a variety of volatiles including various alcohols, terpenes and aromatic compounds. These volatiles can deter insects or other herbivores from feeding, can have direct toxic effects on pests, or they may be involved in recruiting predators and parasitoids in response to feeding damage (Ashour *et al*. 2010). They may also be used by the plants to attract pollinators, protect plants from disease, or they may be involved in interplant communication. As these properties have been known and observed for a very long time, it is a natural progression that three such terpenes, α-terpinene, p-cymene, and d-limonene, have been identified as candidates for biopesticidal use. In the original plant extract the three terpene compounds in combination are the source of insecticidal activity: as this naturally occurring combination is the key active moiety, they are considered and termed to be one active substance. This consideration was agreed at the DG SANCO Phytopharmaceutical Standing Committee meeting 26-27 November 2009 for QRD 420, which contains the same active substance as QRD 460.

The original plant extract (QRD 406) was registered by US EPA as a biopesticide in April 2008. The initial active substance and product was based on a plant extract of *Chenopodium ambrosioides* near *ambrosioides*. The essential oil was harvested from the plant biomass using steam distillation. Variability in growing conditions for the plants meant this active substance suffered from variability in the concentration of the three constituent active terpenes and so an alternative, QRD 460 was developed which is an optimized blend of the three terpenes that reflects the proportions found in the original plant extract QRD 406.

AgraQuest Inc. has submitted this application for approval of the new active substance QRD 460 and its product, QRD 452 respectively, for registration in the EU with ctgb Netherlands as the Rapporteur Member State. It is an insecticide for use on tomatoes and peppers in glasshouses and cucurbits in glasshouses and field at a maximum application rate of 1.523 kg a.s./ha up to 3 times with a 7 day interval between treatments.

Table 6-1: EU Critical GAP for QRD 460 use on Tomatoes, Peppers and Cucurbits

Region	Outdoor/ Protected	Max. No. of Applications	Application Interval (days)	Max. Application		Minimum PHI (days)
				Rate (kg as/ha)	Water (L/ha)	
N EU	Protected	3	7	0.381 – 1.523	400 - 1000	0
S EU	Protected	3	7	0.381 – 1.523	400 - 1000	0
S EU	Outdoor	3	7	0.762 – 1.523	400 - 1000	0

The mode of action of the product is considered non-toxic. Based on laboratory and field trial observations, the mechanism for controlling insect pests is considered to be through degradation of soft insect cuticles resulting in a disruption of insect mobility and respiration. This is considered to occur by direct contact and localized fumigant action. For further details, please refer to document MIII, Section 7, Point 6.

It is noteworthy that these terpenes, α-terpinene, p-cymene, and d-limonene, are commonly used as fragrances and flavourings (Joint FAO/WHO Expert Committee on Food Additives & WHO Technical Report Series 928.). They are present in abundance in many herb plants, and are common in many other edible plants such as citrus fruits, tomatoes, celery and carrots, with various functions as secondary metabolites (Ashour, *et al*, (2010)). Consequently they are a ubiquitous part of both human and animals' natural diet and it is reasonable to expect regular contact with them in the environment without any concern.

All three terpenes are also found, to a greater or lesser extent, in the following EU registered or pending active substances: tea tree oil, thyme oil, orange oil, citronella, spearmint oil, and tagetes (marigold) oil.

Due to the chemical nature of the three terpenes in QRD 460, they disperse rapidly via volatilisation and leave little

to no residues (see Section 4 Metabolism and Residues). Equally they disperse rapidly in the environment into the air and then degrade (see Section 5 Environmental Fate) and so any possible ecotoxicological exposure is expected to be minimal. Additionally, the three terpenes are naturally occurring, are ubiquitous and normal exposure presents no significant risk to humans, animals or the environment, so the plant protection use proposed here adds nothing of significance to the natural exposure, so no additional data other than what is presented here is considered necessary.

The studies presented here under Section 6 Ecotoxicology demonstrate that there are no significant ecotoxicological concerns with regard to the plant protection use of QRD 460 and its product QRD 425 presented here for registration.

To aid evaluation of the dossier, the code designations are described so that it is clear which test substance was used for each study. All substances listed are considered substantially equivalent.

Code Designations
The various AgraQuest code designations that relate to the active substance, products and the submitted documents are as follows:

QRD 406 = *Chenopodium ambrosioides* near *ambrosioides* plant extract technical grade active ingredient (tgai) – consisting of the three terpenes as the active component plus plant derived impurities. Three terpenes comprise approximately 68% of QRD 406.

QRD 400 = formulated EC product with 25% plant extract (QRD 406) active ingredient, 75% other formulants (Also known as FACIN 25EC in some reports and registered in the USA as Requiem® 25EC and Metronome™.) The three terpenes in QRD 400 comprise approximately 17%.

QRD 420 = blended tgai using the three terpenes in the same concentrations as found in QRD 406 with plant derived impurities replaced with canola oil. The three terpenes comprise approximately 67% of QRD 420.

QRD 416 = formulated EC product with 25% blended (QRD 420) a.i., 75% other formulants (same formulants in the same concentrations as QRD 400). The three terpenes comprise approximately 16.75 % of QRD 416.

QRD 452 = QRD 416 – due to a code designation error, the product was re-coded as QRD 452. There are a few studies that reference QRD 416, but the composition is identical to QRD 452. (Also known and registered in the USA as Requiem® EC and Metronome™ EC). The concentration of the three terpenes in QRD 416 and QRD 452 is 16.75%.

QRD 460 = Blended tgai without canola oil. This contains only the three terpenes. The proportions of the three terpenes are essentially the same as the plant extract tgai minus plant derived impurities. So, less QRD 460 is required in Requiem® EC (QRD 452), 16.75% instead of 25%. The percentage of each terpene in QRD 452 and QRD 400 are the same.

IIA 8.1 Avian toxicity

One avian GLP ecotoxicology study has been performed on the original plant extract QRD 406.

The levels of QRD 460 found on plants after application of the product QRD 452 are expected to be minimal due to the rapid volatilisation of the actives, thus exposure of avian species to QRD 460 is not expected to be significant via the oral route or due to contact with treated foliage or fruits. Also due to its rapid volatilisation from water, significant exposure is unlikely to occur to avians from drinking treated water. The only likely exposure could be from air and it is proposed that QRD 460 degrades in air completely in less than 48 hours and so this is also an unlikely route of significant exposure, especially with the main use being in glass houses.

Indeed it should be noted that avians may be subject to greater exposure to α-terpinene, ρ-cymene and d- limonene, from natural plant sources, as they widely occur in nature, particularly in certain fruits. The lack of residue levels after application of QRD 452 and the levels of the terpenes naturally occurring are both addressed more fully under Section 4 Metabolism and Residues.

Due to its rapid volatilisation and degradation, QRD 460 is also not available for avian exposure over a longer period of time and so no chronic studies have been performed, there is no concern for reproduction and no long term risk assessment is considered necessary.

IIA 8.1.1 Acute oral toxicity to a quail species (Japanese or bobwhite), mallard duck, or other bird species

Report:	IIA 8.1.1/01 [redacted] PM & [redacted] JB (2007), QRD 406: An acute oral toxicity study with the Northern Bobwhite, [redacted]. Report Number 489-114, 10 May 2007.

Guidelines

U.S. Environmental Protection Agency. 1996. Series 850-Ecological Effects Test Guidelines (draft), OPPTS Number 850.2100: Avian Acute Oral Toxicity Test.

U.S. Environmental Protection Agency. 1982. Avian Single-Dose Oral LD_{50} Test Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, subsection 71-1. Environmental Protection Agency, Office of Pesticide Programs.

GLP: Yes.

Deviations: Test substance characterisation and stability of the test substance under storage condition at the test site were not determined according to Good Laboratory Practice standards.

The stability, homogeneity and verification of the test substance concentration in the dosing solution were not determined in accordance with Good Laboratorv Practice standards.

Periodic analyses of feed and water for potential contaminants were not conducted according to Good Laboratorv Practice Standards, but were performed using a certified laboratory and standard U.S. Environmental Protection Agency analytical methods.

Executive Summary

The acute oral toxicity of QRD 406 to northern bobwhite (*Colinus virginianus*) was determined. Birds were exposed to a nominal concentration of 2250 mg/kg as a single oral dose. After 14 days no mortality occurred in the control or the test concentration. The LD_{50} was >2250 mg ai/kg.

Materials

Test Material: QRD 406
- **Description:** liquid
- **Lot/Batch No.:** 06D-26
- **Purity:** no data
- **Stability:** not determined
- **Test concentrations:** nominal concentration - 2250 mg/kg bodyweight
- **Vehicle and or positive control:** Corn oil/none

Test animals
- **Species:** Northern bobwhite (*Colinus virginiarus*)
- **Source:** [redacted]
- **Acclimatisation period:** ~6 weeks
- **Treatment for disease:** None during the test
- **Weight:** 196-239 g at test initiation

Feeding:	Game bird ration ad libitum during test. Fasted for 17.5 hours prior to dosing.
Test design	
Replication	2 pens per exposure group
No. of birds per pen	5 males in one pen, 5 females in the other pen
Environmental conditions	
Temperature:	21.9 ± 0.4°C
Relative humidity:	31% ± 10%
Lighting:	8 hours light : 16 hours dark at approximately 178 LuX
Duration of test:	14 days

Study Design and Methods

Experimental dates: 3 to 17 April 2007.

Five female and five male northern bobwhite (*Colinus virginianus*), were assigned to the single treatment group and a control group by indiscriminate draw. The birds were 34 weeks of age and ranged in weight from 196 to 239 g at test initiation. Prior to dosing, birds were acclimated to the study room and caging for approximately six weeks. Throughout acclimation and testing, all test birds were fed a game bird ration ad libitum.

The birds were fasted for approximately 17.5 hours prior to dosing. The nominal single test dosage was 2250 mg/kg bodyweight. The test substance was suspended in corn oil. At test initiation, a single dose of the test substance in diluent was orally intubated directly into the crop or proventriculus of each bird using a stainless steel cannula. All birds received a constant dosage volume of 4 mL/kg body weight.

During acclimation, birds were observed daily, and birds exhibiting abnormal behaviour or physical injury were not used for the test. Following dosing (day 0), birds were observed on multiple occasions with particular attention being paid for signs of regurgitation. Birds were observed at least twice daily for the remainder of the test. Individual body weights were measured on days 0, 3, 7 and 14.

Results and Discussion

There were no mortalities in the control group, and all control birds were normal in appearance and behaviour throughout the test. Additionally, there were no mortalities in the 2250 mg/kg treatment group. There were no significant changes in bodyweight and feed consumption compared with the control after Day 3.

Signs of toxicity were first noted in the 2250 mg/kg treatment group approximately 25 minutes after dosing, when four birds were noted with a loss of coordination. Approximately ten minutes later, all birds in the 2250 mg/kg treatment group were noted with signs of toxicity. Signs of toxicity persisted in at least six birds through the morning of Day 2, and in at least one bird through the afternoon of Day 4. Signs of toxicity noted in the 2250 mg/kg treatment group were loss of coordination and ruffled appearance. Anorexia was also noted among the birds at the 2250 mg/kg treatment group based upon the lack of disturbance of feed from the initial presentation of feed until the morning of Day 1. All birds were normal in appearance from the morning of Day 5 until test termination.

Conclusions

The acute oral LD_{50} value for northern bobwhite exposed to QRD 406 as a single oral dose was determined to be greater than 2250 mg/kg, the highest dosage tested. The no-mortality level was 2250 mg/kg.

([redacted]PM & [redacted]JB, 2007)

IIA 8.1.2 Avian dietary toxicity (5-day) test in a quail species or in mallard duck

Not required for QRD 460 as the active substance dissipates rapidly in the environment from soil and water and does not leave measurable residues in crops from the plant protection use shortly after application, so it is reasonable to conclude that no dietary exposure will occur from this use.

IIA 8.1.3 Avian dietary toxicity (5-day) test in a second unrelated species

Not required for QRD 460 as the active substance dissipates rapidly in the environment from soil and water and does not leave measurable residues in crops from the plant protection use shortly after application, so it is reasonable to conclude that no dietary exposure will occur from this use.

IIA 8.1.4 Subchronic and reproductive toxicity to birds

Not required for QRD 460 as the active substance dissipates rapidly in the environment from soil and water and does not leave measurable residues in crops from the plant protection use shortly after application, so it is reasonable to conclude that no dietary or repeated exposure is expected, therefore no subchronic or reproductive toxicity is likely to occur from this use.

IIA 8.2 Fish toxicity

One fish GLP ecotoxicology study has been performed on QRD 460.

The levels of QRD 460 found in water after application of the product QRD 452 are expected to be minimal due to the rapid volatilisation of the terpene components, therefore, exposure of aquatic species to QRD 460 is not expected to be significant. Selected tests only were carried out with fish to obtain data points for use in models.

It is clear from the physical-chemical properties of the terpene components in QRD 460 (α-terpinene, ρ-cymene, d-limonene) and their fugacity (see Section 5 Environmental Fate and Behaviour) that they are essentially insoluble in water and will volatilise into air where they will degrade rapidly, all of which should mitigate concerns with respect to applications near water.

In a natural water degradation study, the three test items α-terpinene, p-cymene, and d-limonene volatilized from the natural water test systems rapidly with DT_{50}s of 4.1, 11.2 and 3.0 hours and DT_{90}s of 13.7, 37.4, and 10.0 hours for α-terpinene, p-cymene, and d-limonene, respectively.

Due to its rapid volatilisation, QRD 460 is also not available for exposure to aquatic organisms over a longer period of time and so no chronic studies with fish have been performed and no long term risk assessment is considered necessary.

IIA 8.2.1 Acute toxicity of the active substance to fish

IIA 8.2.1.1 Rainbow trout (*Oncorhynchus mykiss*)

The levels of QRD 460 found in water after application of the product QRD 452 are expected to be minimal due to the rapid volatilisation of the terpene components; therefore, exposure of aquatic species to QRD 460 is not expected to be significant.

IIA 8.2.1.2 Warm water fish species

Report:	IIA 8.2.1.2/01 [redacted] M (2011a): QRD 460: Acute toxicity to fathead minnow (*Pimephales promelas*) under flow-through conditions; [redacted]. Study No. 1145.001.523, 11 February 2011.

Guideline

OECD Guideline For Testing Of Chemicals # 203, Fish Acute Toxicity Test (OECD, 1992).

GLP: Yes

Executive Summary:

Following 96 hours of exposure, one dead fish was found in the dilution water control, solvent control and in the 0.218 mg test item/L treatment level. Since not more than one fish had died in the control and solvent control, the test met the validity criterion established by the OECD guideline. No mortality was found in all other concentrations tested. Sublethal effects (complete loss of equilibrium and lethargy) were observed only after 72 hours of exposure in the 0.218, 1.07 and 1.17 mg test item/L concentrations. The mortality and sublethal effects observed were not considered to be test item related, since no fish had died in the three highest test concentrations until test completion.

Groups of fathead minnow (*Pimephales promelas*) were exposed to nominal concentrations of 0.275, 0.55, 1.1, 2.2 and 4.4 mg test item/L in a freshwater flow-through test system for 96 hours at 25 ± 1ºC. In addition fish were maintained in dilution water and 0.1 mL acetone/L flow-through systems for control and solvent control purposes respectively.

The concentration of the test item in the hour 0 solutions ranged from 28.2 to 57.6% of nominal concentrations. The concentration of the test item in the 96-hour solutions ranged from 25.2 to 40.9% of nominal concentrations. The results show that the concentration within each test vessel was maintained during the study.

Based on these results, the 24-, 48-, 72- and 96-hour LC_{50} values were empirically estimated to be > 1.17 mg test item/L. The 96-hour NOEC was determined by visual observation to be 1.17 mg test item/L.

Materials:

Test Material	QRD 460
Description:	Slightly yellow liquid
Lot/Batch #:	Batch No. TL373.81
Purity:	100%
Stability:	expiration date: 27 April 2012
Solubility in water:	Approx 4.5 mg/L
Test concentrations:	Nominal: Control, solvent control and 0.275, 0.55, 1.1, 2.2 and 4.4 mg test item/L Mean measured: Control, solvent control and 0.107, 0.220, 0.382, 1.08, and 1.17 mg test item/L (calculated as arithmetic mean measured concentrations)

Vehicle and/or positive control: Dilution water control (Natural filtered water).
Solvent control – acetone. The solvent concentration in the test vessels was 0.1 mL of acetone per litre

Test animals	Fish
Species:	Fathead minnow (*Pimephales promelas*)
Source:	[redacted]
Acclimatisation period:	Approximately 16 days
Treatment for disease:	None reported
Weight and length of sub-sample from batch of fish at end of exposure period:	Mean length: 25 mm (range 18 to 34 mm); N=30 Mean weight: 0.17 g (range 0.05 to 0.34 g); N=30 Loading: 0.12 g of biomass per liter of test solution

Test design	
Exposure regime:	Flow through
Feeding:	Pretest diet: Flake Food, a dry, commercially available food, generally once daily. Fish were not fed during the 24-hour period prior to test initiation and

	during the exposure period.
Aeration:	Not reported
No of fish per tank:	7

Environmental conditions

Test temperature:	21.8 to 23.2ºC
pH:	8.12 to 8.46
Dissolved oxygen:	8.16 to 8.83 mg/L
Hardness of dilution water:	152 to 156 mg/L CaCO3
Conductivity:	271 to 290 µS/cm
Lighting:	Artificial lighting (353 to 394 Lux, with a 16-hour light, 8-hour dark photoperiod)
Length of test:	96 hours

Study Design and Methods

In-life dates: 22 to 26 July 2010 and the last analytical measurements were completed on 27 July 2010.

The toxicity test was conducted under flow-through (continuous renewal) conditions using an exposure system consisting of a modified proportional diluter, a temperature-controlled water bath and a set of 7 exposure vessels. Based on the results of a preliminary range-finding test definitive test concentrations of 0.275, 0.55, 1.1, 2.2 and 4.4 mg test item/L, a dilution water and solvent (acetone at maximum of 0.1%) control were selected for the definitive exposure.

A 44 mg test item/mL stock solution was prepared prior to test initiation by dissolving 8.8348 g of the test item in 200 mL acetone. Further stock solutions were prepared by sequential dilution. Resulting stock solutions were observed to be clear and colourless, with no undissolved test item. Nominal test concentrations in the test vessels were obtained by adjusting the flow rates of the test item delivery system. All dosing system components which came into contact with test media were constructed entirely out of stainless steel, glass and/or Teflon. For the five treatments, the solvent control and the control the following nominal flow rates were calculated as 0.01 mL/min for stock solution and 100 mL/min for dilution water.

Test vessels were 10 L stainless steel containers. One replicate was maintained for all treatments and the controls, and each vessel was labeled with the test concentration. Test solutions were delivered to the exposure vessels at an approximate rate of 144 L per 24-hour period. This flow-rate is adequate to maintain good water quality and does not stress the fish due to excessive turbulence.

The test item delivery system including the exposure vessels were pre-conditioned with the appropriate test solutions for seven days prior to study initiation. This ensured correct operation of the system and allowed samples of test media to be analyzed to confirm stable test concentrations. Due to the high volatility and poor water solubility of the test item it was not possible to attain the desired nominal concentrations. The highest nominal concentration of 4.4 mg test item/L was chosen based on the solubility of limonene, which has the lowest solubility of the three components and therefore limits the solubility of the formulated test item in water to approximately 4.5 mg/L.

All test vessels were examined at 0, 24, 48, 72 and 96 hours of exposure as follows: mortality was recorded and dead fish were removed. Biological observations, including adverse effects on the exposed fathead minnow, and observations of the physical characteristics of the test solutions (e.g., precipitate, film on the surface of the test solution) were also made and recorded after each 24-hour interval. Effects for this study were based on mortality, defined as the lack of movement by the exposed organisms (i.e., absence of gill movement and reaction to gentle prodding).

Analysis: The solubility of the active ingredients at or near the expected water solubility was determined in a non-GLP preliminary functional water solubility pilot study. The results showed that solution 2 (9.95 mg/L QRD 460) was above the functional solubility. Solution 1 (2.49 mg/L QRD 460) was close the functional solubility.

Prior to the start of the exposure, i.e., day -1, samples from each treatment and control solution were collected and analyzed for QRD 460. During the test, samples were removed at start of exposure and test termination from the test vessels of the controls and the treatment levels for analysis QRD 460. The arithmetic mean measured concentration was first calculated for each active Ingredient.

Three quality control (QC) samples fortified with QRD 460 and a blank control were prepared at each sampling interval at nominal concentrations approximating the test concentrations and remained with the exposure solution sample throughout the analytical process. All exposure solutions and QC samples were analyzed for the active ingredients, (R)-(+) limonene, p-cymene and α-terpinene, by GC-FID based. The method validation established recoveries of 66.6% (RSD 4.12%, N = 5) at a concentration of 0.110 mg test item/L and 60.5% (RSD 3.01%, N = 5) at 1.10 mg test item/L, respectively.

The No-Observed-Effect Concentration (NOEC) during the 96-hour exposure period was determined by visual observation. The NOEC is defined as the highest concentration tested at and below which there were no toxicant-related effects and mortality with respect to the control organisms. The arithmetic mean measured concentrations and the corresponding mortality data derived from the definitive toxicity test were used to estimate the 24-, 48-, 72- and 96-hour median lethal concentration (LC_{50}). The LC_{50} is defined as the concentration of the test item in dilution water which caused mortality of 50% of the test organism population at the stated time interval.

Results and Discussion

The calibration of the stock solution and dilution water pumps showed that the diluter had been functioning properly at least 24 hours before experimental start. Recoveries of the test solution samples performed on day -1 ranged from 47.5 to 66.7% of the nominal fortified levels. Due to fast degradation and/or volatilization of the active ingredients it is not possible to obtain higher recoveries in the diluter system. Additionally, the highest concentration is close to the water solubility limit which makes the occurrence of surface films more likely and therefore leads to even lower recoveries.

The concentration of the test item in the hour 0 solutions ranged from 28.2 to 57.6% of nominal concentrations. The concentration of the test item in the 96-hour solutions ranged from 25.2 to 40.9% of nominal concentrations. The results show that the concentration within each test vessel was maintained during the study. Analysis of the QC samples resulted in measured concentrations similar to the recoveries in the method validation and therefore demonstrated that for all concentrations, satisfactory precision and quality control were maintained during the analysis of exposure solutions.

Based on these results, the arithmetic mean measured test concentrations of 0.107, 0.220, 0.382, 1.08 and 1.17 mg test item/L were used for the evaluation of the biological data.

Table IIA 8.2.1.2-1. Mean measured concentrations of QRD 460 measured in the exposure solutions of the 96-hour exposure of fathead minnow (*Pimephales promelas*).

Nominal Concentration (mg test item/L)	Arithmetic Mean Measured Concentration (mg test item/L)				Recovery (%)[b]
	(R)-(+) limonene	p-cymene	α-terpinene	Sum	
Control	<LOQ	<LOQ	<LOQ	<LOQ	NA
Solvent control	<LOQ	<LOQ	<LOQ	<LOQ	NA
0.275	0.0157[a]	0.0396	0.0517[a]	0.107	38.9
0.55	0.0378	0.0669	0.115	0.220	40.0
1.1	0.0626	0.140	0.179	0.382	34.7
2.2	0.181	0.404	0.499	1.08	49.3
4.4	0.189	0.447	0.538	1.17	26.7

LOQ Limit of Quantification. Determined as 0.110 mg test item/L (corresponding to 0.0197 mg (R)-(+) limonene/L, 0.0246 mg p-cymene/L and 0.0657 mg α-terpinene/L).
NA Not Applicable.

a Value below LOQ (only approximate value) used to calculate arithmetic mean.
b Recovery calculated from the sum of the three active ingredients which make up for 100% in the test item.

Following 96 hours of exposure, one dead fish was found in the dilution water control, solvent control and in the 0.220 mg test item/L treatment level. Since not more than one fish had died in the control and solvent control, the test met the validity criterion established by the OECD guideline. No mortality was found in all other concentrations tested. Sublethal effects (complete loss of equilibrium and lethargy) were observed only after 72 hours of exposure in the 0.220, 1.08 and 1.17 mg test item/L concentrations. The mortality and sublethal effects observed were not considered to be test item related, since no fish had died in the three highest test concentrations during the test. No changes in the characteristics of the test solutions were observed throughout the duration of the test.

Based on these results, the 24-, 48-, 72- and 96-hour LC_{50} values were empirically estimated to be > 1.17 mg test item/L. The 96-hour NOEC was determined by visual observation to be 1.17 mg test item/L.

Table IIA 8.2.1.2-2: Cumulative percent mortality during the 96-hour exposure of fathead minnow (*Pimephales promelas*) to QRD 460.

Arithmetic Mean Measured Concentration (mg test item/L	Cumulative Percent Mortality			
	24-Hour	48-Hour	72-Hour	96-Hour
Control	0	0	14	14
Solvent control	0	14	14	14
0.107	0	0	0	0
0.218	0	0	0	14
0.371	0	0	0	0
1.07	0	0	0	0
1.17	0	0	0	0

Conclusion

Based on these results, the 24-, 48-, 72- and 96-hour LC_{50} values were empirically estimated to be > 1.17 mg test item/L. The 96-hour NOEC was determined by visual observation to be 1.17 mg test item/L.

([redacted]M, 2011a)

IIA 8.2.1.3 Acute toxicity of metabolites, degradation or reaction products to the more sensitive of the fish species used to test the acute toxicity of the active substance.

Due to the low toxicity of QRD 460, and its rapid volatilization and breakdown in air, there is no significant risk of exposure of fish species to the metabolites, degradation or reaction products.

IIA 8.2.2 Chronic toxicity to fish

Due to the low toxicity of QRD 460, and its rapid volatilization and breakdown in air the potential for chronic exposure to fish is minimal and does not require further consideration.

IIA 8.2.3 Chronic toxicity (28 day exposure) to juvenile fish growth and behaviour

Due to the low toxicity of QRD 460, and its rapid volatilization and breakdown in air, the potential for chronic exposure to fish is minimal and does not require further consideration.

IIA 8.2.4 Fish early life stage toxicity test

Due to the low toxicity of QRD 460, and its rapid volatilization and breakdown in air, the potential for early life stage exposure is minimal. Also, the constituents of the active substance, terpenoid blend (α-terpinene, ρ-cymene, d- limonene) QRD 460, are well described chemically, have low toxicity, are naturally occurring, and show no potential for endocrine disruption. Therefore, it is reasonable to conclude that a fish early life toxicity test is not necessary.

IIA 8.2.5 Fish life cycle test

Due to the low toxicity of QRD 460, and its rapid volatilization and breakdown in air, the potential for exposure is minimal. Also, the constituents of the active substance, terpenoid blend (α-terpinene, ρ-cymene, d- limonene) QRD 460, are well described chemically, have low toxicity, are naturally occurring, and show no potential for endocrine disruption. Therefore, it is reasonable to conclude that a fish early life toxicity test is not necessary.

IIA 8.2.6 Bioconcentration in fish

IIA 8.2.6.1 Bioconcentration potential of the active substance in fish

The log P_{ow} of the QRD 460 constituents are as follows
α-terpinene Log P_{ow} = 5.09
ρ-cymene Log P_{ow} = 5.08
d- limonene Log P_{ow} = 4.85

Log P_{ow} values greater than 3 suggest the possibility of bioconcentration, however it is clear from Section 5 Environmental Fate that these terpenes because of their high volatility combined with their water insolubility will not have sufficient residence time in water to provide significant exposure to fish or other aquatic organisms to trigger any meaningful risk. It is also reasonable to conclude that naturally occurring substances such as these will not have a propensity to bioaccumulate or bioconcentrate in aquatic organisms

IIA 8.2.6.2 Bioconcentration potential of metabolites, degradation and reaction products

The log P_{ow} of the QRD 460 constituents are as follows
α-terpinene Log P_{ow} = 5.09
ρ-cymene Log P_{ow} = 5.08
d- limonene Log P_{ow} = 4.85

Log P_{ow} values greater than 3 suggest the possibility of bioconcentration, however it is clear from Section 5 Environmental Fate that these terpenes because of their high volatility combined with their water insolubility will not have sufficient residence time in water to provide significant exposure to fish or other aquatic organisms to trigger any meaningful risk. It is also reasonable to conclude that naturally occurring substances such as these will not have a propensity to bioaccumulate or bioconcentrate in aquatic organisms

IIA 8.2.7 Aquatic bioavailability/biomagnification/depuration

This is not an EC requirement.

IIA 8.3 Toxicity to aquatic species other than fish and aquatic species field testing

There is not considered to be sufficient exposure in water to warrant any concern.

IIA 8.3.1 Acute toxicity to aquatic invertebrates

It should be noted that due to the high volatility and low water solubility of QRD 460 it was not possible to attain the desired nominal concentrations. The highest nominal concentration of 4.4 mg test item/L was chosen based on the solubility of limonene, which has the lowest solubility of the three components and therefore limits the solubility of the formulated test item in water to approximately 4.5 mg/L.

The difficulties encountered in the aquatic studies due to the physical/chemical properties of QRD 460 are additional reasons why actual exposure during use of the plant protection product is expected to be so low as to pose insignificant risk to aquatic organisms.

IIA 8.3.1.1 Acute toxicity (24 and 48 hour) for *Daphnia* preferably (*Daphnia magna)*

Report:	IIA 8.3.1.1 [redacted]M (2011b) QRD 460: Acute toxicity to Water Fleas (*Daphnia magna*) under flow-through conditions; [redacted], [redacted]. Study No. 1145.001.110, February 2011

Guideline

OECD Guideline for Testing of Chemicals # 202, Daphnia sp. Acute Immobilization Test (OECD, 2004).

GLP: Yes

Executive Summary

Daphnia magna less than 24 hours old at the start of the test, were exposed to a series of concentrations of QRD-460 in a flow through test system. Based on the results of a preliminary range-finding test, definitive test concentrations of 0.275, 0.55, 1.1, 2.2 and 4.4 mg test item/L, a dilution water and solvent (acetone) control. The mean measured test concentrations were 0.0991, 0.132, 0.341, 0.565 and 1.04 mg test item/L (calculated as arithmetic mean measured concentrations).

After 24 hours and 48 hours of exposure, no immobilization was observed in the solvent control and the 0.0883 and 0.132 mg test item/L treatment levels. Immobilization of 5, 15, 25 and 45% was observed in the dilution water control and the 0.338, 0.540 and 1.02 mg test item/L treatment levels. Sublethal effects (lethargy, swimming carrying, erratic and floaters) were observed in the 0.338, 0.540 and 1.02 mg test item/L treatment levels starting at hour 24. One daphnid in the solvent control was observed to be swimming carrying after 48 hours of exposure.

Based on these results, the 24- and 48-hour EC_0 values were empirically estimated to be > 1.04 mg test item/L. The 48-hour NOEC was determined to be 0.132 mg test item/L.

Materials

Test Material

Description:	Slightly yellow liquid
Lot/Batch #:	Batch No. TL373.81
Purity:	100%
Stability:	expiration date: 27 April 2012
Density	Not reported

Treatments

Test concentrations:	Based on the results of a preliminary range-finding test, definitive test concentrations of 0.275, 0.55, 1.1, 2.2 and 4.4 mg test item/L, a dilution water and solvent (acetone) control
Vehicle and/or positive control:	Natural filtered water

Test animals

Species:	Daphnia magna
Source:	[redacted]

[redacted]) culture

Culture medium: natural filtered water

Feeding: 2.0 mL of a solution containing approximately 4 x 107 cells/mL of the unicellular green alga, Ankistrodesmus falcatus (ANK) and four drops or 0.5 mL of a combination of yeast, cereal leaves and flaked fish food (YCT) daily. The daphnids were not fed during the 48 hour exposure period.

Test design

Test vessels: glass battery jars having a total volume capacity of 1.6 L.

Test medium: natural filtered water

Exposure regime: Flow through

Aeration: No additional aeration

Replication: Yes, 4 replicates from each concentration and controls.

Environmental conditions

Test temperature: 19.3 to 21.1 °C

pH: 8.28 to 8.41

Dissolved oxygen: 7.12 to 8.53 mg/L

Water hardness 150 mg/L as $CaCO_3$

Conductivity of dilution water 300 µS/cm

Lighting: 362 to 454 lux with a 16-hour light, 8-hour dark photoperiod

Length of test: 48 hours

Study Design and Methods

In-life dates: 02 to 04 November 2010. Last analytical work was performed on 05 November 2010

Daphnia magna less than 24 hours old at the start of the test, were exposed to a series of concentrations of QRD 460 in a flow through test system at nominally 20 ± 1°C. The exposure concentrations for this study were determined based on the result of a preliminary range finding test. Definitive test concentrations were 0.275, 0.55, 1.1, 2.2 and 4.4 mg test item/L, a dilution water and solvent (acetone) control.

A 44 mg test item/mL stock solution was prepared prior to test initiation by dissolving 22.00514 g of the test item in 500 mL acetone. Further stock solutions were prepared. All resulting stock solutions were observed to be clear and colourless with no undissolved test item. Nominal test concentrations in the test vessels were obtained by adjusting the flow rates of the test item delivery system. For the solvent control vessels, a stock solution with acetone only was used (limited to 0.1%) and for the control vessels, dilution water only was used. Four replicates were maintained for all treatments and the controls.

A constant-flow test item delivery system equipped with membrane pumps was used for test item stock solution and dilution water delivery. For the five treatments, the solvent control and the control the following nominal flow rate was calculated to be 50 mL/min for all exposures. Based on the calibrated flow of dilution water the flow-splitting chambers cycled 360 times per 24 hours to provide 12.86 volume replacements per day. This flow-rate is adequate to maintain good water quality and does not stress the daphnids due to excessive turbulence. Test vessels were glass battery jars having a total volume capacity of 1.6 L. The test item delivery system including the exposure vessels were pre-conditioned with the appropriate test solutions for at least 18 days prior to study initiation. This ensured correct operation of the system and allowed samples of test media to be analyzed to confirm stable test concentrations.

Due to the high volatility and poor water solubility of the test item it was not possible to attain the desired nominal concentrations. The highest nominal concentration of 4.4 mg test item/L was chosen based on the solubility of limonene, which has the lowest solubility of the three components and therefore limits the solubility of the formulated test item in water to approximately 4.5 mg/L.

The test was initiated when daphnids were impartially distributed to each of the four replicates for each nominal concentration and the controls. The number of immobilized daphnids observed in each replicate test vessel was recorded at test initiation and after 24 and 48 hours of exposure. Biological observations (e.g., abnormal behavior or appearance of the test organisms) and observations of the physical characteristics of the test solutions (e.g., precipitate, film on the surface of the test solution) were also made and recorded after 0, 24 and 48 hours of exposure. The pH, dissolved oxygen (DO) concentration and temperature were measured at 0, 24 and 48 hours in one replicate of each treatment level and the controls. Continuous temperature monitoring was performed in an additional vessel adjacent to the test vessels throughout the exposure period.

Analysis: All exposure solutions and QC samples were analyzed for the active ingredients, (R)-(+) limonene, p-cymene and α-terpinene, by GC-FID at 0 and 48 hours. The method validation established recoveries of 66.6% (RSD 4.12%, N= 5) at a concentration of 0.110 mg test item/L and 60.5% (RSD 3.01%, N= 5) at 1.10 mg test item/L, respectively. The arithmetic mean measured concentration (CMean) was calculated for each active Ingredient ((R)-(+) limonene, p-cymene and α-terpinene).

The solubility of the active ingredients at or near the expected water solubility was determined in a non-GLP preliminary functional water solubility pilot study. A stock solution of QRD 460 in acetone was prepared at a concentration of 99.5 mg/mL. A 10-mL sample was taken and extracted with 2 mL hexane and the concentration of (R)-(+) limonene, p-cymene and α-terpinene in the extract analysed by GC-FID. The results show that solution 2 (9.95 mg/L QRD 460) was above the functional solubility. Solution 1 (2.49 mg/L QRD 460) was close the functional solubility.

The arithmetic mean measured concentrations and the corresponding immobilization data derived from the definitive toxicity test were used to estimate the 24- and 48-hour median effect concentration (EC_{50}). The EC_{50} is defined as the concentration of the test item in dilution water which caused immobilization of 50% of the test organism population at the stated time interval. Since immobilization was below 50% throughout the duration of the test, the EC_{50} values were empirically estimated to be greater than the highest test concentration tested.

The No-Observed-Effect Concentration (NOEC) was determined by visual observation and is defined as the highest concentration tested at which there was no toxicant-related immobilization or physical and behavioral abnormalities (e.g., lethargy) with respect to the control organisms.

Results: QRD-460 in the test solutions were close to nominal in both tests at 0 hours (between 21.3 and 35.1%) and 48 hours (between 18.8 and 41.2%). Mean measured concentrations over the test period ranged from 23.2 to 32.1% (see Table IIA 8.3.1.1-1).

Table IIA 8.3.1.1-1: Mean measured concentrations of QRD 460 measured in the exposure solutions of the 48-hour exposure *Daphnia magna*

Nominal concentration (mg test item/L)	Arithmetic Mean Measured Concentration (mg test item/L)				Recovery (%)[b]
	(R)-(+) limonene	p-cymene	α-terpinene	Sum	
Control	<LOQ	<LOQ	<LOQ	<LOQ	NA
Solvent Control	<LOQ	<LOQ	<LOQ	<LOQ	NA
0.275	0.0117[a]	0.0307	0.0488[a]	0.0911[a]	33.1
0.55	0.0177[a]	0.0448	0.0699	0.132	24.1
1.1	0.0511	0.109	0.181	0.341	31.0
2.2	0.0778	0.188	0.300	0.565	25.7
4.4	0.148	0.335	0.561	1.04	23.7

LOQ Limit of Quantification. Determined as 0.110 mg test item/L (corresponding to 0.0197 mg (R)-(+) limonene/L, 0.0246 mg p-cymene/L and 0.0657 mg α-terpinene/L).
NA Not Applicable.
a Value below LOQ (only approximate value) used to calculate arithmetic mean.
b Recovery calculated from the sum of the three active ingredients which make up for 100% in the test item.

Results and Discussion

After 24 hours and 48 hours of exposure, no immobilization was observed in the solvent control and the 0.0911 and 0.132 mg test item/L treatment levels. Immobilization of 5, 15, 25 and 45% was observed in the dilution water control and the 0.341, 0.565 and 1.04 mg test item/L treatment levels. Sublethal effects (lethargy, swimming carrying, erratic and floaters) were observed in the 0.341, 0.565 and 1.04 mg test item/L treatment levels starting at hour 24. One daphnid in the solvent control was observed to be swimming carrying after 48 hours of exposure. The 48-hour NOEC, based on sublethal effects, was determined to be 0.132 mg test item/L.

No changes in the characteristics of the test solutions were observed throughout the duration of the test.

The EC_{50} is defined as the concentration resulting in a 50% reduction in the number of live Daphnia within the test period. The mean EC50 values for 24 and 48 hours were >1.04 mg/L (mean measured concentration), respectively.

Conclusion

Based on these results, the 24- and 48-hour EC_{50} values were empirically estimated to be >1.04 mg test item/L (mean measured concentration).

([redacted]M, 2011b)

IIA 8.3.1.2 Acute toxicity (24 and 48 hour) for representative species of aquatic insects

Due to the low toxicity and rapid volatilization of the active substance QRD 460 into air, the potential for acute exposure to aquatic insects is minimal and does not require further consideration. In addition, from the *Daphnia* test above, it was not possible to make the active substance soluble enough in water to set a meaningful endpoint for the EC_{50} value, therefore it is reasonable to conclude that significant exposure to other aquatic organisms is unlikely.

IIA 8.3.1.3 Acute toxicity (24 and 48 hour) for representative species of aquatic crustaceans (species unrelated to *Daphnia*)

Due to the low toxicity and rapid volatilization of the active substance QRD 460 into air, the potential for acute exposure to aquatic crustaceans is minimal and does not require further consideration. In addition, from the *Daphnia* test above, it was not possible to make the active substance soluble enough in water to set a meaningful endpoint for the EC_{50} value, therefore it is reasonable to conclude that significant exposure to other aquatic organisms is unlikely.

IIA 8.3.1.4 Acute toxicity (24 and 48 hour) for representative species of aquatic gastropod molluscs

Due to the low toxicity and rapid volatilization of the active substance QRD 460 into air, the potential for acute exposure to aquatic gastropod molluscs is minimal and does not require further consideration. In addition, from the *Daphnia* test above, it was not possible to make the active substance soluble enough in water to set a meaningful endpoint for the EC_{50} value, therefore it is reasonable to conclude that significant exposure to other aquatic organisms is unlikely.

IIA 8.3.2 Chronic toxicity to aquatic invertebrates

IIA 8.3.2.1 Chronic toxicity in *Daphnia magna* (21-day)

Report:	IIA 8.3.2.1/01 [redacted] M 2011c, QRD 460: Chronic reproduction test with daphnids (*Daphnia magna*) under flow-through conditions, [redacted] Study Number 1145.003.231, May 2011

Guidelines

OECD Guideline # 211 *Daphnia magna* Reproduction Test (OECD, 2008)

GLP: Yes

Executive Summary

The chronic toxicity of QRD 460 to *Daphnia magna* was determined. Organisms were exposed to five nominal concentrations (0.1, 0.2, 0.4, 0.8 and 1.6 mg test item/L), a dilution water control and a solvent control. These corresponded to mean measured concentrations of 0.0424, 0.0756, 0.173, 0.214 and 0.361 mg test item/L respectively. Mean measured concentrations were used for the reporting of results. There was no significant difference in survival among daphnids exposed to any treatment level when compared to the control. A significant reduction in offspring per female among daphnids exposed to the 0.361 mg test item/L treatment level when compared to the pooled controls. The EC_{50} value for reproduction was 0.309 mg test item/L. . Statistical analysis demonstrated a significant reduction in body length of daphnids exposed to the 0.214 and 0.361 mg test item/L treatment levels when compared to the pooled controls.

Based on the most sensitive endpoint (length), the NOEC and LOEC values for the reproduction study were 0.173 and 0.214 mg test item/L, respectively.

Materials

Test Material:	QRD 460
Description:	Not Reported
Lot/Batch #:	TL373.81
Purity:	Confidential, see Document J
Stability of test compound:	Expiry 27 April 2012
Test concentrations:	Control, solvent control and 0.1, 0.2, 0.4, 0.8 and 1.6 mg test item/L (nominal)
Vehicle and/or positive control:	Elendt M4 medium
Analysis of test concentrations:	Yes
Test animals	
Species:	*Daphnia magna*
Source:	[redacted]
Treatment for disease:	None Reported
Feeding:	green alga, *Ankistrodesmus falcatus* (ANK) and a combination of yeast, cereal leaves and flaked fish food (YCT) daily
Test design	
Exposure regime:	Flow through (flow of dilution water the flow-splitting chambers cycled 360 times per 24 hours to provide 12.86 volume replacements per day)
Aeration:	No
Replication:	Four
No of *Daphnia* per test concentration:	Ten

Environmental conditions	
Test temperature:	Continuously measured temperature: 19 to 21oC Single-point measured temperature: 19 to 22ºC
pH:	6.90 to 8.03
Dissolved oxygen:	6.82 to 9.09 mg/L
Lighting:	316 to 443 Lux, with a 16-hour light, 8-hour dark photoperiod
Length of test:	21 days

Study Design and Methods

Experimental dates: 17 February to 11 March 2011

Test procedure and apparatus

Test vessels were glass battery jars having a total volume capacity of 1.6 L. Exposure solutions drained from each vessel through two 2-cm holes, approximately 15 cm from the bottom of the jars, which maintained the test solution volume at 1.4 L. The drain holes were covered with a Nitex® 40-mesh screen to prevent loss of the daphnids. Four replicates were maintained for all treatments and the controls. At test start, the animals were less than 24 hours old. During the definitive test, from day 0 to day 20, the daphnids were fed 6 times per day using 3 mL food suspension containing approximately 4 x 10^7 cells/mL of the unicellular green alga, *Ankistrodesmus falcatus* (ANK) per feeding interval and replicate, i.e., 18 mL food suspension per day and replicate. In addition, 0.1 mL of a combination of yeast, cereal leaves and flaked fish food (YCT) was fed daily from days 0 to 6. From days 7 to 11, 0.5 mL YCT was fed. From days 12 to 20, 1 mL YCT was fed. Based on the calibrated flow of dilution water the flow-splitting chambers cycled 360 times per 24 hours to provide 12.86 volume replacements per day. This flow-rate was adequate to maintain good water quality and did not stress the daphnids due to excessive turbulence. The test temperature was 19 to 22ºC. A photoperiod of 16 hours light: 8 hours dark, was provided.

Preparation of test solutions

Based on the results of a preliminary range-finding test, definitive test concentrations of 0.1, 0.2, 0.4, 0.8 and 1.6 mg test item/L, a dilution water and solvent control were selected for the definitive exposure. Stock solutions were prepared by the addition of a known quantity of test item to acetone solution as follows:

Weight of Test Item (g)	Diluted to Volume (mL) with acetone	Stock Solution Concentration (mg test item/mL)
3.2	200	16
1.6	200	8
0.8	200	4
1	500	2

In addition, a stock solution was prepared at a concentration of 1 mg test item/mL by diluting 100 mL of the 2 mg test item/mL stock solution to 200 mL with acetone. The resulting stock solutions were observed to be clear and colorless. Nominal test concentrations in the test vessels were obtained by adjusting the flow rates of the test item delivery system. For the solvent control, a stock solution containing only acetone was prepared. For the dilution water control only dilution water without test item and solvent was used.

Analytical method

Two days prior to the start of the definitive exposure, samples were removed from each treatment level and the controls and analyzed for QRD 460 concentration. Results were used to judge whether sufficient quantities of the test item was being delivered to the test vessels and whether the appropriate test concentrations were being maintained in order to initiate the definitive exposure. During the in-life phase, water samples were removed from

one replicate of each treatment level, dilution water control and solvent control and analyzed for QRD 460 concentration on test days 0, 5, 12 and 21. Three quality control (QC) samples fortified with QRD460 and a blank control were prepared at each sampling interval at nominal concentrations approximating the test concentrations and remained with the exposure solution sample throughout the analytical process. All exposure solutions and QC samples were analyzed for the active ingredients, (R)-(+) limonene, p-cymene and α-terpinene, by GC-FID based on the method validated prior to the definitive test.

Observations of effects

The number of immobilized adult daphnids, number of surviving females and males and observations of abnormal behavior were recorded daily. Assessments of offspring released were determined on test days 8, 11, 13, 15, 18, 20 and 21. The number of immobilized offspring and the time to first brood release were recorded for each replicate vessel. At test completion (day 21), total body length of each surviving adult daphnid was measured.

Physical and chemical parameters

The dissolved oxygen (DO) concentrations, pH and temperature were measured and recorded in each test vessel at experimental start and weekly thereafter until test termination (day 21). In addition, the pH, DO concentration and temperature were measured daily in one vessel of each test concentration and the controls. The temperature was also continuously monitored in one replicate throughout the study. Total hardness, alkalinity and specific conductivity were monitored at experimental start and on test days 5, 12 and 19 in one replicate of the highest treatment level and the dilution water control during the exposure.

Results and Discussion

Analytical data

The nominal concentrations for the definitive test were 0.1, 0.2, 0.4, 0.8 and 1.6 mg test item/L. The mean measured concentrations were 0.0424, 0.0756, 0.173, 0.214 and 0.361 mg test item/L, corresponding to 14.0 to 63.2% % of the nominal concentrations. Although the recoveries were lower than nominal they were generally consistent between sampling intervals and within treatment levels. An appropriate dose gradient was maintained. The mean measured concentrations were used for reporting the results.

Biological data

The results are summarised in the Table below.

Table IIA 8.3.1.2-1: Summary of effects of long-term exposure of QRD 460 on *Daphnia magna*

Time weighted Concentration (mg test item/L)	Mean Survival (%)	Mean # of Living Offspring Released/Female	SD	Mean Total Body Length (mm) of Female Daphnids	SD
Control	85	137	26.7	4.70	0.12
Solvent Control	92	109	8.0	4.50	0.14
Pooled Controls	89	123	23.7	4.60	0.16
0.0424	85	115	22.9	4.51	0.21
0.0756	88	134	18.6	4.61	0.08
0.173	78	122	41.4	4.48	0.25
0.214	75	113	30.1	4.31*	0.10
0.361	50	38*	10.0	3.95*	0.07

SD Standard Deviation.

* Significantly reduced when compared to the control, based on Bonferroni t-Test ($p < 0.05$).

There were several male daphnia present in the test population during this study. The presence of males in a normal culture population is not uncommon and did not negatively impact the evaluation of the measured endpoints.

After 21 days of exposure, survival in the dilution water control and solvent control was 85% and 92%, respectively. The mean cumulative number of offspring released per female during the test was 137 and 109 for the dilution water control and solvent control, respectively. The dilution water control and solvent control organisms released their first brood of offspring on exposure day 8. Survival of 85.0, 88.0, 78.0, 75.0 and 50.0% was observed among daphnids exposed to the 0.0424, 0.0756, 0.173, 0.214 and 0.361 mg test item/L treatment levels, respectively. Statistical analysis (Anova Test) determined no significant difference in survival among daphnids exposed to any treatment level when compared to the control. First brood release among daphnids exposed to the 0.0424, 0.0756, 0.173 and 0.214 mg test item/L treatment levels was observed between test days 8 and 11, which were consistent with the dilution water control and solvent control performance. In the highest test concentration, 0.361 mg test item/L, first brood release was observed between days 10 and 18.

Daphnids exposed to the 0.0424, 0.0756, 0.173, 0.214 and 0.361 mg test item/L treatment levels released a mean number of cumulative live offspring per female of 115, 134, 122, 113 and 38, respectively. Statistical analysis (Bonferroni t-test) determined a significant reduction in offspring per female among daphnids exposed to the 0.361 mg test item/L treatment level when compared to the pooled controls (mean number of cumulative live offspring per female: 123). The EC_{50} value for reproduction was 0.309 mg test item/L with a 95% confidence interval of 0.284 to 0.327 mg test item/L. After 21 days of exposure, the mean total body length among female daphnids exposed to the dilution water control, solvent control and the 0.0424, 0.0756, 0.173, 0.214 and 0.361 mg test item/L treatment levels averaged 4.70, 4.50, 4.51, 4.61, 4.48, 4.31 and 3.95 mm, respectively. Statistical analysis (Bonferroni t-test) demonstrated a significant reduction in body length exposed to the 0.214 and 0.361 mg test item/L treatment levels when compared to the pooled controls (4.60 mm).

Based on the most sensitive endpoint (length), the NOEC and LOEC values for the reproduction study were 0.173 and 0.214 mg test item/L, respectively.

Physical and chemical data

The results of the water quality measurements made during this study established that conditions maintained throughout the 21-day exposure were satisfactory for the promotion of survival, reproduction and growth (total body length) of *Daphnia magna*. The single point temperature was between 19 and 22°C, whereas the continuously measured temperature ranged from 19 to 21°C throughout the exposure. The pH ranged from 6.90 to 8.03 and the DO concentration from 6.82 and 9.09 mg/L. Light intensity of the test area ranged from 316 to 443 lux at test start and completion.

Conclusions

Based on the most sensitive endpoint (length), the NOEC and LOEC values for the reproduction study were 0.173 and 0.214 mg test item/L, respectively.

(dXe炮j/5 M 2011c)

IIA 8.3.2.2 Chronic toxicity for representative species of aquatic insects

Due to the low toxicity and rapid volatilization of the active substance QRD 460 into air, the potential for chronic exposure to aquatic organisms is minimal and does not require further consideration.

IIA 8.3.2.3 Chronic toxicity for representative species of aquatic gastropod mollusc

Due to the low toxicity and rapid volatilization of the active substance QRD 460 into air, the potential for chronic exposure to aquatic organisms is minimal and does not require further consideration.

IIA 8.3.3 Aquatic field testing

Due to the low toxicity and rapid volatilization of the active substance QRD 460 into air, the potential for chronic exposure to aquatic organisms is minimal and does not require further consideration.

IIA 8.4 Effects on algal growth and growth rate (2 species)

QRD 460 has very low toxicity, rapidly volatilizes, and breaks down quickly in air after volatilisation. From Section 5 Environmental Fate it has been shown that the three components α-terpinene, ρ-cymene, d- limonene are not persistent and dissipate in a matter of hours. As they are also highly insoluble and volatile, they do not remain in water very long after application as a plant protection product and, as such, their use is unlikely to result in any significant exposure of aquatic plants such as algae. On this basis, no algal studies have been performed as the likelihood of exposure is sufficiently small.

IIA 8.5 Effects on sediment dwelling organisms

QRD 460 has very low toxicity, rapidly volatilizes, and breaks down quickly in air after volatilisation. From Section 5 Environmental Fate it has been shown that the three components α-terpinene, ρ-cymene, d-limonene are not persistent and dissipate in a matter of hours. As they are also highly insoluble and volatile, they do not remain in water very long after application as a plant protection product and, as such, their use is unlikely to result in any significant exposure of sediment dwelling organisms. However, a study on *Chironomus riparius* has been performed and is presented here.

The difficulties encountered in the aquatic studies due to the physical/chemical properties of QRD 460 are additional reasons why actual exposure during use of the plant protection product is expected to be so low as to pose insignificant risk to aquatic organisms.

IIA 8.5.1 Acute test

Report:	IIA 8.5.1/01 [redacted] M (2011d), QRD 460: Acute toxicity to midge larvae (*Chironomus riparius*) under flow-through conditions [redacted]. Study No. 1145.001.175, 11 February 2011.

Guidelines

OECD Guideline for Testing of Chemicals # 202, Daphnia sp. Acute Immobilization Test (OECD, 2004).

GLP: Yes

Executive Summary

The acute toxicity of QRD 460 to *Chironomus riparius* was determined. Third instar larvae were exposed to a range of nominal concentrations of 0.275, 0.55, 1.1, 2.2 and 4.4 mg test item/L alongside a dilution water control and solvent control. The measured concentrations were 0.122, 0.257, 0.360, 0.657 and 0.953 mg test item/L (calculated as arithmetic mean measured concentrations).

After 24 hours, midge larvae of all treatment levels were burrowed in the sand. Following 48 hours of exposure, immobilization of 10, 10, 5, 20, 5, 30 and 70% was observed in the dilution water control, solvent control and the 0.122, 0.257, 0.360, 0.657 and 0.953 mg test item/L treatment levels. Due to the low immobilization in the next higher treatment level, the 20% immobilization of the 0.257 mg test item/L treatment level is not considered test item related.

Based on these results, the 48-hour EC_{50} value was calculated to be 0.86 mg test item/L (95% confidence interval: 0.75 – 0.93 mg test item/L). The 48-hour NOEC was determined to be 0.360 mg test item/L.

Materials

Test Material: QRD 460

Lot/Batch #: Batch No. TL373.81

Purity: 100%

Description: Slightly yellow liquid

Stability of test compound: Stable; expiration date: 27 April 2012

Treatments

Test concentrations: Control, solvent control and 0.275, 0.55, 1.1, 2.2 and 4.4 mg test item/L corresponding to mean measured test item concentrations of 0.122, 0.250, 0.360, 0.657 and 0.953 mg test item/L (calculated as arithmetic mean measured concentrations).

Solvent: Acetone (0.1%)

Analysis of test concentrations: Yes at 0 and 48 hours (based on measurement of QRD 460)

Test organisms:

Species: *Chironomus riparius*

Age: 2-3 day old first-instar larvae (L1)

Source: [redacted]

Feeding: During culture, the midge larvae were generally fed a solution containing approximately 4 x 107 cells/mL of the unicellular green alga *Ankistrodesmus falcatus* (ANK). The feeding rate was adjusted to meet the nutritional requirements of the midge larvae which varied depending upon their stage of development. The midge larvae were fed 0.25 mL of a finely ground fish flake suspension (10 mg/mL) after test initiation but not were not fed during the 48 hour test period.

Test design:

Test vessels: glass battery jars having a total volume capacity of 1.6 L

Test medium: natural filtered water

Replication: 4 replicates of 5 chironomids

Exposure regime: Flow through

Duration: 48 hours

Environmental conditions

Test temperature: 19.7 to 20.1°C

pH range: 8.03 to 8.42

Dissolved oxygen: 6.74 to 9.10 mg/L

Total hardness of dilution water: 148 mg/L CaCO3

Lighting: 349 to 432 lux with a 16-hour light, 8-hour dark photoperiod

Study Design and Methods

Experimental dates: 17 to 19 November 2010. Last analytical work was performed on 20 November 2010.

The toxicity test was conducted under flow-through (continuous renewal) conditions using an exposure system consisting of a modified proportional diluter, a temperature-controlled water bath and a set of 28 exposure vessels.

Based on the results of a preliminary range-finding test, definitive test concentrations of 0.275, 0.55, 1.1, 2.2 and 4.4 mg test item/L, a dilution water and solvent control (acetone 0.1%) were selected for the definitive exposure.

A 44 mg test item/mL stock solution was prepared prior to test initiation by dissolving 8.8072 g of the test item in 200 mL acetone. Further stock solutions were prepared by serial dilution. All resulting stock solutions were observed to be clear and colourless, with no undissolved test item. Nominal test concentrations in the test vessels were obtained by adjusting the flow rates of the test item delivery system. For the solvent control vessels, a stock solution with acetone only was used. For the control vessels, dilution water only was used.

A constant-flow test item delivery system equipped with membrane pumps was used for test item stock solution and dilution water delivery. All dosing system components which came into contact with test media were constructed entirely out of stainless steel, glass and/or Teflon. Based on the calibrated flow of dilution water the flow-splitting chambers cycled 360 times per 24 hours to provide 12.86 volume replacements per day. This flow-rate is adequate to maintain good water quality and does not stress the midge larvae due to excessive turbulence. The flow of the dilution water was verified 12 and 5 days prior to test initiation. A visual check of the diluter was performed twice daily for the duration of the test.

Test vessels were glass battery jars having a total volume capacity of 1.6 L. Exposure solutions drained from each vessel through two 2-cm holes, approximately 15 cm from the bottom of the jars, which maintained the test solution volume at 1.4 L. Four replicates were maintained for all treatments and the controls.

The test item delivery system including the exposure vessels were pre-conditioned with the appropriate test solutions for one day prior to study initiation. Due to the high volatility and poor water solubility of the test item it was not possible to attain the desired nominal concentrations. The highest nominal concentration of 4.4 mg test item/L was chosen based on the solubility of limonene, which has the lowest solubility of the three components and therefore limits the solubility of the formulated test item in water to approximately 4.5 mg/L

The number of immobilized midge larvae observed in each replicate test vessel was recorded daily during the 48-hour exposure. Due to the sand in each test vessel, non-visible organisms were recorded as burrowed (B). Additional to the test solution observation at test completion, the sand was checked for midge larvae. Missing larvae were presumed as immobilized.

Analysis: The solubility of the active ingredients at or near the expected water solubility was determined in a non-GLP preliminary functional water solubility pilot study. The results show that solution 2 (9.95 mg/L QRD 460) was above the functional solubility. Solution 1 (2.49 mg/L QRD 460) was close the functional solubility.

Prior to the start of the exposure phase, i.e., day -1, samples from one replicate of the treatment solutions and control solutions were collected and analyzed for QRD 460. Results of the pretest analyses were used to judge whether sufficient quantities of the test item were being delivered to the test vessels and whether the appropriate test concentrations were being maintained in order to initiate the definitive exposure. During the test, samples were removed at start of exposure and test termination from the test vessels of the controls and the treatment levels for analysis QRD 460. The arithmetic mean measured concentration was calculated for each active ingredient.

The No-Observed-Effect Concentration (NOEC) was determined by visual observation and is defined as the highest concentration tested at which there was no toxicant-related immobilization or physical and behavioral abnormalities (e.g., lethargy) with respect to the control organisms.

The arithmetic mean measured concentrations and the corresponding immobilization data derived from the definitive toxicity test were used to calculate the 48-hour median effect concentration (EC_{50}). The EC_{50} is defined as the concentration of the test item in dilution water which caused immobilization of 50% of the test organism population at the stated time interval. Prior to statistical analyses, the data were arc sine (square root) transformed. A T-test was used to compare the performance of the dilution water control organisms with that of the solvent control organisms. Analyses established no significant difference between the dilution water control and solvent control. Statistical comparisons to determine treatment effects were performed utilizing pooled control data and the EC_{50} value was calculated using TOXSTAT® version 3.5.

Results and Discussion

The results of the test solution analysis for the active ingredient of QRD 460 are summarized in Table IIA 8.5.1-1. Recoveries of the test solution samples performed on day -1 ranged from 22.1 to 40.3% of the nominal fortified levels. Due to fast degradation and/or volatilization of the active ingredients it is not possible to obtain higher recoveries in the diluter system. Additionally, the highest concentration is close to the water solubility limit which makes the occurrence of surface films more likely and therefore leads to even lower recoveries. Although the recoveries were low, the test was started since the diluter system had been shown to function correctly.

The concentration of the test item in the hour 0 solutions ranged from 20.9 to 47.9% of nominal concentrations. The concentration of the test item in the 48-hour solutions ranged from 22.4 to 47.4% of nominal concentrations. The results show that the concentration within each test vessel was maintained during the study. Based on these results, the arithmetic mean measured test concentrations of 0.122, 0.257, 0.360, 0.657 and 0.953 mg test item/L were used for the evaluation of the biological data.

Analysis of the QC samples resulted in measured concentrations similar to the recoveries in the method validation. Therefore, it was demonstrated that for all concentrations satisfactory precision and quality control were maintained during the analysis of exposure solutions

Table IIA 8.5.1-1: Mean measured concentrations of QRD 460 measured in the exposure solutions of the 48-hour exposure of midge larvae (*Chironomus riparius*).

Nominal Concentration (mg test item/L)	Arithmetic Mean Measured Concentration (mg test item/L)				Recovery (%)[a]
	(R)-(+) limonene	p-cymene	α-terpinene	Sum	
Control	<LOQ	<LOQ	<LOQ	<LOQ	NA
Solvent Control	<LOQ	<LOQ	<LOQ	<LOQ	NA
0.275	0.0218	0.0313	0.0686	0.122	44.3
0.55	0.0444	0.0667	0.146	0.257	46.7
1.1	0.0514	0.107	0..202	0.360	32.7
2.2	0.0856	0.205	0.366	0.657	29.9
4.4	0.128	0.323	0.502	0.953	21.7

LOQ Limit of Quantification. Determined as 0.110 mg test item/L (corresponding to 0.0197 mg (R)-(+) limonene/L, 0.0246 mg p-cymene/L and 0.0657 mg α-terpinene/L).
NA Not Applicable.
a Recovery calculated from the sum of the three active ingredients which make up for 100% in the test item.

The mean measured concentrations, the corresponding percent immobilization and observations recorded during the 48-hour test are presented in Table IIA 8.5.1-2. Due to the low immobilization in the next higher treatment level, the 20% immobilization of the 0.257 mg test item/L treatment level is not considered test item related. No changes in the characteristics of the test solutions were observed throughout the duration of the test.

Based on these results, the 48-hour EC_{50} value was calculated to be 0.86 mg test item/L (95% confidence interval: 0.75 – 0.93 mg test item/L). The 48-hour NOEC was determined to be 0.360 mg test item/L.

Table IIA 8.5.1-2: Cumulative percent mortality during the 48-hour exposure of midge larvae (*Chironomus riparius*) to QRD 460.

Arithmetic mean measured concentration (mg test item/L)	Cumulative Immobilization (%)	
	24-Hour	48-Hour
Control	0	10
Solvent Control	0	10
0.121	0	5
0.257	0	20[a]
0.358	0	5
0.648	0	30
0.952	0	70

a Due to the low immobilization in the next higher treatment level, this immobilization is not considered test item related.

Conclusions

Based on these results, the 48-hour EC_{50} value was calculated to be 0.86 mg test item/L (95% confidence interval: 0.75 – 0.93 mg test item/L). The 48-hour NOEC was determined to be 0.360 mg test item/L.

([redacted]M, 2011d)

IIA 8.5.2 Chronic test

Due to the low toxicity and rapid volatilization of the active substance QRD 460 into air, the potential for chronic exposure to aquatic organisms is minimal and does not require further consideration. In addition the acute test results do not suggest significant toxicity concern.

IIA 8.6 Effects on aquatic plants

QRD 460 has very low toxicity, rapidly volatilizes, and breaks down quickly in air after volatilisation. From Section 5 Environmental Fate it has been shown that the three components α-terpinene, ρ-cymene, d-limonene are not persistent and dissipate in a matter of hours. As they are also highly insoluble and volatile, they do not remain in water very long after application as a plant protection product and, as such, their use is unlikely to result in any significant exposure of aquatic plants by the proposed use pattern.

IIA 8.7 Effects on bees

QRD 460 has very low toxicity, rapidly volatilizes, and breaks down quickly in air after volatilisation. From Section 5 Environmental Fate it has been shown that the three components α-terpinene, ρ-cymene, d-limonene are not persistent and dissipate in a matter of hours. It should be noted that as bees forage in many plants that naturally contain the terpene components of QRD 460, exposure to these compounds is likely quite normal in the life of a bee.

As QRD 460 rapidly volatilises, no acute oral study have been performed as it is more likely that bees would come into contact with QRD 460 during spraying or when foraging on recently treated plants. Consequently contact studies have been performed.

One acute contact study has been conducted using QRD 420 and one acute contact study has been conducted with the formulation QRD 452.

Both studies demonstrated a lack of toxicity at the highest levels tested.

IIA 8.7.1 Acute oral toxicity

See above.

IIA 8.7.2 Acute contact toxicity

Report:	IIA 8.7.2/01. [redacted] JR & [redacted] HO (2009a). QRD 420: An Acute Contact Toxicity Study with the Honeybee, [redacted]. Project Number: 489-115, August 2009.

Guidelines

U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines (draft), OPPTS Number 850.3020: Honeybee, Acute Contact Toxicity

OECD Guidelines for the Testing of Chemicals, 214: Honeybees, Acute Contact Toxicity Test

EPPO Guideline 170, Guideline on Test Methods for Evaluating the Side-Effects of Plant Protection Products on Honey Bees.

GLP: Yes.

Executive Summary

Young adult worker honey bees were exposed to five doses of QRD 420 ranging from 6.25 to 100 micrograms per bee (µg a.i./bee) administered topically in a droplet to the abdomen and/or thorax of each bee. Observations of mortality and other signs of toxicity were made twice within the first four hours of dosing and then at approximately 24 and 48 hours after test initiation. The cumulative mortality observed in the test groups was used to determine the LD_{50}.

The 48-hour acute contact LD_{50} value for honey bees exposed to QRD 420 was determined to be greater than 100 µg a.i./bee.

Materials:

Test Material	QRD 420
Description:	Pale amber liquid
Lot/Batch No.:	Lot Number Y001
Purity:	100%
Stability:	Stable
Control:	Two control groups, one treated with acetone, the other with water
Treatment doses:	Test Substance Doses: 6.25, 12.5, 25.0, 50.0 and 100 µg a.i./bee
Toxic standard:	Dimethoate Positive Control Doses: 0.05, 0.10 and 0.30 µg a.i. dimethoate/bee
Administration:	Cuticular absorption following the application of droplets (2 µL) to the dorsal body surface
Test organisms	
Species:	Young adult worker honey bees (*Apis mellifera*) (Hymenoptera: Apidae)
Source:	[redacted]
Food:	50 % aqueous sucrose solution
Test Design	
Test cage description:	Stainless steel cylinders (approx. 9 cm in diameter x 9 cm high). Each chamber

	was covered by a petri dish.	
Replication:	Contact test:	3 replicates
No. of bees/replicate:	20	

Environmental test conditions

Temperature:	25 ± 2°C
Humidity:	50 to 70%
Photoperiod:	Complete darkness
Duration of test:	48 h

Study Design and Methods

Experimental dates: July 21, 2009 to July 23, 2009

Young adult worker honey bees were exposed to five doses of QRD 420 ranging from 6.25 to 100 µg a.i./bee administered topically in a droplet to the abdomen and/or thorax of each bee. A negative control group and a solvent control group were maintained concurrently. Three replicate test chambers were maintained in each control and treatment group, with 20 bees in each test chamber.

Test procedures: QRD 420 was dissolved in acetone. Bees were anaesthetised and treated individually by topical application of 2 µL of test item solution, control and reference item solution were applied dorsally to the thorax of each bee, respectively. After application, the bees were returned to the test cages and fed with a 50% aqueous sucrose solution *ad libitum*.

Mortality and sublethal effects were assessed twice within the first 4 hours and then at approximately 24 and 48 hours after dosing.

Results and Discussion

For QRD 420 treatments, mortality at the end of the test ranged from 1.7 to 13.3%. Surviving bees in the treatment groups appeared normal throughout the test with the exception of two immobile bees in the 25.0 µg/bee QRD 420 treatment group. Based on these results, the 48-hour LD50 for honey bees topically exposed to QRD 420 was determined to be greater than 100 µg/bee. The mortality was 13.3% or less in all of the treatment groups at test termination and did not occur in a dose-responsive pattern. Therefore, the NOEC was determined to be 100 µg/bee; the highest concentration of QRD 420 tested.

Mean mortality in the negative control and solvent control groups was 6.7 and 1.7%, respectively, at test termination. Mean mortality at 24 hours after test initiation in the 0.05, 0.10 and 0.30 µg dimethoate/bee groups was 1.7, 18.3 and 91.7%, respectively. Some hyperactive bees were noted in the 0.10 and 0.30 µg dimethoate/bee groups on the day of test initiation.

The 24-hour LD_{50} value for honey bees exposed to dimethoate in this test was determined to be 0.153 µg/bee, with 95% confidence limits of 0.134 and 0.177 µg/bee. This value was within the desired range of 0.10 to 0.30 µg/bee and served to confirm that the procedures used to administer the dose were effective. At test termination the mean mortality in the 0.05, 0.10 and 0.30 µg dimethoate/bee groups was 23.3, 28.3 and 91.7%, respectively.

Conclusion

The 48-hour acute contact LD_{50} value for honey bees exposed to QRD 420 was determined to be greater than 100 µg/bee.

([redacted] JR & [redacted] HO, 2009a)

Report:	IIA 8.7.2/02. [redacted] JR & [redacted] HO (2009b). QRD 452: An Acute Contact Toxicity Study with the Honeybee, [redacted]. Project Number: 489-116, August 2009.

Guidelines

U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines (draft), OPPTS Number 850.3020: Honeybee, Acute Contact Toxicity

OECD Guidelines for the Testing of Chemicals, 214: Honeybees, Acute Contact Toxicity Test

EPPO Guideline 170, Guideline on Test Methods for Evaluating the Side-Effects of Plant Protection Products on Honey Bees.

GLP: Yes.

Executive Summary

Young adult worker honey bees were exposed to five doses of QRD 452 ranging from 6.25 to 100 micrograms per bee (µg/bee) administered topically in a droplet to the abdomen and/or thorax of each bee. Observations of mortality and other signs of toxicity were made twice within the first four hours of dosing and then at approximately 24 and 48 hours after test initiation. The cumulative mortality observed in the test groups was used to determine the LD_{50}.

The 48-hour acute contact LD_{50} value for honey bees exposed to QRD 452 was determined to be greater than 100 µg/bee.

Materials

Test Material	QRD 452
Description:	Pale amber liquid
Lot/Batch No.:	Lot Number R001
Purity:	Technical grade 25% w/w Terpenes and inerts: 75% w/w
Stability:	Stable
Control:	Two control groups, one treated with acetone, the other with water
Treatment doses:	Test Substance Doses: 6.25, 12.5, 25.0, 50.0 and 100 µg/bee
Toxic standard:	Dimethoate Positive Control Doses: 0.05, 0.10 and 0.30 µg a.i. dimethoate/bee
Administration:	Cuticular absorption following the application of droplets (2 µL) to the dorsal body surface
Test organisms	
Species:	Young adult worker honey bees (*Apis mellifera*) (Hymenoptera: Apidae)
Source:	[redacted]
Food:	50 % aqueous sucrose solution
Test Design	
Test cage description:	Stainless steel cylinders (approx. 9 cm in diameter x 9 cm high). Each chamber was covered by a petri dish.
Replication:	3 replicates
No. of bees/replicate:	20

Environmental test conditions

Temperature:	25 ± 2°C
Humidity:	50 to 70%
Photoperiod:	Complete darkness
Duration of test:	48 h

Study Design and Methods

Experimental dates: July 28, 2009 to July 30, 2009

Young adult worker honey bees were exposed to five doses of QRD 452 ranging from 6.25 to 100 µg/bee administered topically in a droplet to the abdomen and/or thorax of each bee. A negative control group and a solvent control group were maintained concurrently. Three replicate test chambers were maintained in each control and treatment group, with 20 bees in each test chamber.

Test procedures: QRD 452 was dissolved in acetone. Bees were anaesthetised and treated individually by topical application of 2 µL of test item solution, control and reference item solution were applied dorsally to the thorax of each bee, respectively. After application, the bees were returned to the test cages and fed with a 50% aqueous sucrose solution *ad libitum*.

Mortality and sublethal effects were assessed twice within the first 4 hours and then at approximately 24 and 48 hours after dosing.

Results and Discussion

For QRD 452 treatments, mortality at the end of the test ranged from 1.7 to 8.3%. Overall surviving bees in the treatment groups appeared normal throughout the test. Based on these results, the 48-hour LD_{50} for honey bees topically exposed to QRD 452 was determined to be greater than 100 µg/bee. The mortality was 8.3% or less in all of the treatment groups at test termination and did not occur in a dose-responsive pattern. Therefore the NOEC was determined to be 100 µg/bee, the highest concentration tested.

Mean mortality in the negative control and solvent control groups was 8.3 and 6.7%, respectively, at test termination. Mean mortality at 24 hours after test initiation in the 0.05, 0.10 and 0.30 µg dimethoate/bee groups was 8.3, 25.0 and 96.7%, respectively. Some hyperactive bees were noted in the 0.10 and 0.30 µg dimethoate/bee groups on the day of test initiation. The 24-hour LD50 value for honey bees exposed to dimethoate in this test was determined to be 0.14 µg/bee, with 95% confidence limits of 0.1 and 0.3 µg/bee. This value was within the desired range of 0.10 to 0.30 µg dimethoate/bee and served to confirm that the procedures used to administer the dose were effective. At test termination the mean mortality in the 0.05, 0.10 and 0.30 µg dimethoate/bee groups was 23.3, 31.7 and 100%, respectively.

Conclusion

The 48-hour acute contact LD_{50} value for honey bees exposed to QRD 452 was determined to be greater than 100 µg/bee.

([redacted] JR & [redacted] HO, 2009b)

IIA 8.7.3 Toxicity of residues on foliage to honey bees

This is not an EC requirement and in any case, residues do not remain on treated foliage long enough to pose a risk to honey bees.

IIA 8.7.4 Bee brood feeding test

Not required as no significant toxicity detected in the acute tests.

IIA 8.8 Effects on non-target terrestrial arthropods

QRD 460 has very low toxicity, rapidly volatilizes, and breaks down quickly in air after volatilisation. From Section 5 Environmental Fate it has been shown that the three components α-terpinene, ρ-cymene, d- limonene are not persistent and dissipate in a matter of hours. There is a possibility of exposure of non-target terrestrial arthropods and so a number of studies have been performed using QRD 460 on the aphid parasitoid *Aphidius rhopalosiphi*, the predatory mite, *Typhlodromus pyr*i, the predatory bug*, Orius laevigatus* and the plant dwelling insect *Coccinella septempunctata*.

In the four studies performed, no significant toxicity was observed and the LR_{50} of QRD 460 was >200.00 L a.s./ha for each time interval tested i.e. 10x higher than the field rate showed no significant effect.

In all four studies performed, the ER_{50} was >200.00 L of a.s./ha at each time interval tested and the NOEC for reproduction was 200.00 L of a.s./ha.

IIA 8.8.1 Using artificial substrates

IIA 8.8.1.1 Parasitoid

Report:	IIA 8.8.1.1/01 [redacted] M (2010a). Effects of QRD 460 on the aphid parasitoid *Aphidius rhopalosiphi* de stefani perez (Hymenoptera: Braconidae) under laboratory conditions. [redacted]. Final Report BT074/10, 10 December 2010.

Guidelines

The principles of the study were based on the ESCORT I Guidance Document (Barrett et al., 1994), the ESCORT II Guidance Document (Candolfi et al., 2000), the IOBC Guidelines (Mead-Briggs et al., 2000) and the guideline of the ring testing group (Mead-Briggs et al., 2009). Data on toxicity to *Aphidius rhopalosiphi* will be produced in compliance with the EU Registration directive 91/414/EEC (amended by the Commission Directive 96/12/EC).

GLP: Yes.

Executive Summary

The effects of residues of QRD 460 on the mortality and reproduction of the aphid parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae), were tested with a laboratory study.

The results of the study show that the test item, QRD 460, when applied to glass surfaces, caused no statistically significant mortality in the test organism at any time interval after introduction into the treated cages.

The LR_{50} of the test material was > 200.00 L of a.s./ha for each time interval tested.

During the reproductive test, exposure to the test item did not result in a significant difference in parasitisation capacity of the surviving wasps when compared with the control. The ER_{50} was >200.00 L of a.s./ha at each time interval tested.

The NOEC for the reproduction was 200.00 L of a.s./ha.

Materials

Test Material:	QRD 460
Description:	Technical active ingredient for an EC insecticide formulation
Lot/Batch No.:	AQ421-13-2
Purity:	100%
Density:	Not reported
Stability:	Stable
Control:	Deionised water
Toxic standard:	Perfekthion EC (nominally 400 g dimethoate/L) in purified water, applied at a rate of 10 mL product/ha.
Spray volume rate:	200 L spray solution/ha
Application method:	Automatic Potter Spray Tower
Test rates:	200 L a.s./ha

Test organisms

Species	*Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae)
Source:	[redacted]
Food:	1:3 v/v solution of honey and water
Age at test start:	adults

Test design

Arenas:	circular glass plates (diameter: 10.00 cm), which were assembled into cages with an aluminium frame (diameter: 10.00 cm; height: 1.50 cm; width: 1.00 cm) after the spray layer dried.
Replication:	3
No. of wasps/arena:	10

Environmental test conditions

Temperature:	exposure phase: 20.67 - 21.67°C / reproduction phase: 19.00 - 21.33°C
Humidity:	exposure phase: 62.00 - 70.00%
Photoperiod:	16 h light and 8 h darkness reproduction phase: 4000 - 19800 lux
Duration of test:	30 days

Study Design and Methods

Experimental dates: 15th November 2010 - 15th December 2010

The objective of the study was to evaluate the potential adverse effects from residues of the test item QRD 460 after application to glass plates under laboratory conditions. The effects of the test substance on the survival and reproduction performance of the aphid parasitoid *Aphidius rhopalosiphi* were assessed under laboratory conditions. The test was carried out as single rate limit test and, in order to demonstrate the rapid degradation of the test item, the test system was inserted into the treated cages at five time intervals.

In order to evaluate the mortality of the parasitoids, adults, less than 48 hours old, were exposed to dried spray deposit of the test item on glass plates. In order to confirm the test system efficacy, Perfekthion (a.s. Dimethoate) was applied as reference substance, while deionised water was used as the control. The glass surfaces were treated with undiluted test item and the parasitoids were added at five different time intervals: within 1 hour, 2 hours, 4 hours, 6 hours and 24 hours after the spray application. The applications were performed by a laboratory sprayer, calibrated to deliver spray at volume rate equivalent to 200 L/ha. Simultaneously other glass plates were sprayed with deionised water for the control and with Perfekthion for the reference substance group. After the application of the spray solutions and when the treated plates had dried the exposure units were assembled.

The condition of the exposed parasitoids was assessed after 2, 24 and 48 hours of their introduction into the treated cages.

After 48 hours of exposure to the treated glass plates, the surviving females were removed with an aspirator from the exposure cage. Each female (15 females for each treatment group and 15 females for the control group) was transferred to a single fecundity cage, and given a 24 hour time period to parasitize aphids. In the fecundity cages there were untreated Aphid infested plants. After 24 hours, the female parasitoids were removed from the fecundity cages and their condition (alive, dead or not recovered) was recorded. The parasitized aphids within the fecundity arenas were left to develop in situ and the number of aphid "mummies" that developed was recorded 12 days later.

Results and Discussion

The mean mortality and fecundity results are given in Table IIA 8.8.1.1-1.

The test item QRD 460 had no effect on the behaviour of the treated parasitoids as demonstrated by the lack of any changes in the normal behaviour. The treated parasitoids showed no signs of reduced coordination and no difference in the general activity with respect to the wasps of the control group.

The corrected mortality of the treated parasitoids was zero at each time interval tested, which indicates that the test item is harmless at the tested rate against the aphid parasitoid *Aphidius rhopalosiphi*. The LR_{50} of the product QRD 460, was > 200 L of a.s./ha for each time interval tested.

Results indicate no statistical difference in the reproduction performance of any of the QRD 460 treatment groups when compared with the control group. Each time interval tested was similar to the control by the Dunnett's test. The ER_{50} was > 200 L of a.s./ha for each time interval tested.

Table IIA 8.8.1.1-1: Effects of QRD 460 on mortality and fecundity of *Aphidius rhopalosiphi*

Treatment rate – L a.s./ha	% mortality	Mean corrected % mortality	Number of mummies/female	% effect on reproduction compared to control
0	10.00	n.a.	8.00	n.a.
200 (1 hr AT)	10.00	0.00	7.87	1.67
200 (2 hr AT)	3.33	0.00	7.40	7.50
200 (4 hr AT)	10.00	0.00	6.47	19.17
200 (6 hr AT)	6.67	0.00	9.93	-24.17
200 (24 hr AT)	3.33	0.00	7.00	12.50
Reference substance (3.65 g a.s./ha)	100.00	100.00	-	-

AT After treatment

In accordance with IOBC/WSPR guidance, the test is valid because the mortality observed in the control group after 48 hours of exposure was ≤13.00% and the mortality caused by the reference item was ≥50%. Moreover the control parasitisation rate (mean) was >5 aphid mummies per surviving female and only one female failed to produce mummies.

Conclusions

The results of the study show that the test item, QRD 460, when applied to glass surfaces, caused no statistically significant mortality in the test organism, *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera: Braconidae), at any time interval after introduction into the treated cages.

The LR_{50} of the test material was >200.00 L of a.s./ha for each time interval tested.

During the reproductive test, exposure to the test item did not result in a significant difference in parasitisation capacity of the surviving wasps when compared with the control. The ER_{50} was > 200.00 L of a.s./ha at each time

interval tested.

The NOEC for the reproduction was 200.00 L of a.s./ha.

([redacted]M, 2010a)

IIA 8.8.1.2 Predatory mites

Report:	IIA 8.8.1.2/01. [redacted]M (2010b), Effects of QRD 460 on the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) under Laboratory Conditions [redacted]. Final Report BT074/10, 19 November 2010

Guidelines

Blümel *et al*. (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products.

GLP: Yes.

Executive Summary

QRD460 (100% active ingredient), was tested in a worst-case exposure laboratory dose-response study, to determine the effects on the predatory mite *Typhlodromus pyri*.

The results obtained at the end of the study show that the test item, QRD 460, when applied to glass surfaces, caused statistically significant mortality when the test organism, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae), was added to the treated surfaces immediately after spray treatment (1 and 2 hours after treatment). Mortality in the mites added 4, 6, and 24 hours after spray treatment was not statistically different from control mortality.

The LR_{50} of the test product was evaluated at 7 days post-treatment. The LR_{50} of the test material was > 200.00 L of a.s./ha for each time interval tested.

During the reproductive test, exposure to the test item did not result in significant difference in reproductive capacity of the surviving mites when compared with the controls. The ER_{50} was >200.00 L of a.s./ha at each time interval tested.

The NOEC for the reproduction was 200.00 L of a.s./ha.

Materials

Test Material:	QRD 460
Description:	Technical active ingredient for an EC insecticide formulation
Lot/Batch No.:	AQ421-13-2
Purity:	100%
Density:	Not reported
Stability:	Stable (expiry date August 2012)
Control:	Deionised water
Toxic standard:	Perfekthion EC (nominally 400 g dimethoate/L) in purified water, applied at a rate of 10 mL product/ha.
Test rates:	200 L/ha (equivalent to 200 L a.s./ha)
Spray volume rate:	200 L/ha
Application method:	Potter tower Maximum exposure was achieved by treating all inner sides of the cages with the test products

Test organisms

Species: *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae)

Source: [redacted]).

Food: During the tests, the mites were fed with pollen and Tetranichus urticae, and were provided with water ad libitum

Age at test start: protonymphs

Test design

Arenas: A test unit consisted of two cover slides, which were placed on top of a piece of moist filter paper. The two cover slides were fixed together by a glass bar glued on them in the horizontal direction. In the arena, an area (that measured approximately 10 -13 cm2) was bordered by a barrier of a non-drying glue gel to prevent mites from escaping.

Replication: 3

No. of mites/arena : 20

Environmental test conditions

Temperature: 23.00-27.00°C

Humidity: 61.00-84.00% RH

Photoperiod: 16 h light and 8 h darkness

Duration of test: 14 days, 7 for mortality phase and 7 for fecundity phase.

Study Design and Methods

Experimental dates: 14th to 29th October 2010

In order to evaluate the mortality of the mites, protonymphs were exposed to dried spray deposit of the test item on glass surface. The glass surfaces were treated with undiluted test item and the mites were added at five different time intervals: within 1 hour, 2 hours, 4 hours, 6 hours and 24 hours after the spray application. The applications were performed by a laboratory sprayer, calibrated to deliver spray at volume rate equivalent to 200 L/ha. Simultaneously other cages were sprayed with deionised water for the control and with Perfekthion for the reference substance group.

Three replicates per treatment group, each containing 20 predatory mites were tested. The mortality and escape rate of the mites were assessed on day 4 and day 7 after exposure. On day 7, the sex ratio was determined. Reproduction per female was recorded from day 7 to day 14.

Reproduction performance was assessed in all test groups where the corrected mortality was ≤50%.

Results and Discussion

Mortality and fecundity are summarized in Table IIA 8.8.1.2-1.

Mortality in mites added to the treated cages 1 and 2 hours after spray treatment was greater than that of the controls: means for trials 2 and 3 significantly different from control mean at alpha = 0.05 by Dunnett's test. Mortality in the mites added 4, 6, and 24 hours after spray treatment was not statistically different from control mortality. The LR_{50} of the product QRD 460 evaluated at 7 days after treatment, was > 200 L of a.s./ha for each time interval tested.

Results indicate no statistical difference in the reproduction performance of any of the QRD 460 treatment groups when compared with the control group. Each time interval tested was similar to the control by the Dunnett's test. The ER_{50} was > 200 L of a.s./ha for each time interval tested.

Table IIA 8.8.1.2-1: Effects of QRD 460 on mortality and fecundity of *Typhlodromus pyri*

Treatment rate – L a.s./ha	% mortality	Mean corrected % mortality	Mean eggs/female	% effect on reproduction compared to control
0	18.33	Na	5.29	na
200 (1 hr AT)	45.00	32.65	5.75	-8.72
200 (2 hr AT)	46.67	34.69	4.27	19.24
200 (4 hr AT)	33.33	18.37	6.52	-23.32
200 (6 hr AT)	26.67	10.20	3.86	27.10
200 (24 hr AT)	23.33	6.12	6.54	-23.74
Reference substance (3.65 g a.s./ha)	93.33	91.84	-	-

AT After treatment

According to IOBC guideline, the test is valid because the mortality observed in the control group 7 days after treatment was ≤20%. Moreover, the mean mortality (control corrected) caused by the reference substance was between 50% and 100%. The cumulative mean number of eggs per female in the control group (from day 7 to day 14) was ≥4.

Conclusions

The results of the study show that the test item, QRD 460, under the conditions of this test, caused a statistically significant increase in mortality when the test organism *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) was added to the test apparatus immediately after spray treatment (within 1 hour) and 2 hours after spray treatment when compared to controls. Mortality in the mites added 4, 6, and 24 hours after spray treatment was not statistically different from control mortality.

The LR_{50} of the test product was evaluated at 7 days post-treatment. The LR_{50} of the test material was > 200.00 L of a.s./ha for each time interval tested.

During the reproductive test, exposure to the test item did not result in significant difference in reproductive capacity of the surviving mites when compared with the controls. The ER_{50} was > 200.00 L of a.s./ha at each time interval tested.

The NOEC for the reproduction was 200.00 L of a.s./ha.

([redacted]M, 2010b)

IIA 8.8.1.3 Ground dwelling predatory species (selected to be relevant to the intended uses of preparations)

Not considered relevant for the intended use of QRD 460.

IIA 8.8.1.4 Foliage dwelling predatory species (selected to be relevant to the intended uses of preparations)

Report:	IIA 8.8.1.4/01 [redacted]M (2010c), Effects of QRD 460 on the predatory bug, *Orius laevigatus* (Fieber (Heteroptera: Anthocoridae) under Laboratory Conditions, [redacted]. Final Report BT077/10, 10 December 2010

Guidelines

Bakker *et al*. (2000). A laboratory test for evaluating the effects of plant protection products on the predatory bug, *Orius laevigatus* (Fieber) (Heteroptera, Anthocoridae).

GLP: Yes.

Executive Summary

QRD460 (100% active ingredient), was sprayed on glass surfaces undiluted at 200 L as/ha and the insects (second instar nymphs) were added at five different time intervals (1, 2, 4, 6 and 24 hours after spray treatment). The survival of bugs was assessed at day 9 (when 80% had become adults). The fecundity of female bugs was evaluated over two consecutive 2-day periods. Fertility was evaluated at day 21 on the first batch of eggs.

The results of the study show that QRD 460 applied to glass surfaces caused statistically significant mortality to *Orius laevigatus*, when they were exposed one hour after spraying the test item. Mortality of juvenile bugs added 2, 4, 6 or 24 hours after spray treatment was not significantly different to untreated controls.

The LR_{50} of QRD 460 was >200.00 L as/ha for each time interval tested.

During the reproduction test exposure to QRD 460 did not result in a significant difference in reproductive capacity compared to the controls.

The ER_{50} was >200.00 L as/ha for each time interval tested and the NOEC for reproduction was 200.00 L as/ha.

Materials

Test Material: QRD 460
- **Description:** Technical active ingredient for an EC insecticide formulation
- **Lot/Batch No.:** AQ421-13-2
- **Purity:** 100%
- **Density:** Not reported
- **Stability:** Stable
- **Control** Deionised water
- **Toxic standard** Perfekthion EC (393 g dimethoate/L), applied at a rate of 10 g product/ha in deionised water
- **Test rates:** 200 L/ha (equivalent to 200 L a.s./ha)
- **Spray volume rate:** 200 L/ha
- **Application method:** Potter tower

Test organisms
- **Species:** *Orius laevigatus* Fieber (Heteroptera: Anthocoridae)
- **Source:** [redacted]
- **Food:** *Ephis spp.*, Lepidoptera eggs
- **Age at test start:** Second instar nymphs

Test design
- **Arenas:** Glass container (5cm diameter, 3cm height), inner walls coated with talcum to prevent the nymphs climbing. The top of the container was closed with gauze,
- **Replication:** 8
- **No. nymphs/arena:** 10

Environmental test conditions
- **Temperature:** 21.33-27.33ºC
- **Humidity:** 53.00-74.00% RH
- **Photoperiod:** 16 h light and 8 h darkness
- **Duration of test:** 21 days in total

Study Design and Methods

Experimental dates: 19th October – 10th November 2010

Orius laevigatus second instar nymphs were exposed to dried QRD460 residues on a glass surface. QRD 460 was applied undiluted at 200 L as/ha and nymphs were added 1, 2, 4, 6 or 24 hours later.

On day 9 of the test, when at least 80% of the individuals in the control were adult, the number of survivors and the number of dead bodies were counted. Fecundity assessments started on day 14. 15 females per treatment were selected for determination of oviposition. Individual females were confined to the oviposition substrate and their egg production was assessed for two consecutive 2-day intervals. Total number of eggs produced per female over the 4-day period was recorded. The substrate containing the first batch of eggs were stored for five more days (day 21) and assessed again for numbers of hatched or not hatched eggs.

Results and Discussion

Mortality and fecundity are summarised in Table IIA 8.8.1.4-1.

Table IIA 8.8.1.4-1: Effects of QRD 460 on mortality and fecundity of *Orius laevigatus*

Treatment rate – L a.s./ha	% mortality	Mean corrected % mortality	No. eggs/female/ day	% hatching rate	% effect on reproduction compared to control
0	17.50	na	3.67	74.91	na
200 (1 hr AT)	46.25	34.85	2.43	66.78	10.85
200 (2 hr AT)	18.75	1.52	3.05	77.32	-3.22
200 (4 hr AT)	22.50	6.06	3.30	73.23	2.22
200 (6 hr AT)	21.25	4.55	3.00	76.34	-1.91
200 (24 hr AT)	22.50	6.06	2.95	81.67	-9.02
Reference substance (3.65 g a.s./ha)	93.75	92.42	--		--

AT After treatment

Mortality in bugs added one hour after spray treatment was statistically significantly greater ($\alpha = 0.05$) than that of the controls. For all remaining time intervals mortality was not statistically different from the controls. The LR_{50} for QRD 460 evaluated 9 days after treatment was >200 L as/ha for each time interval tested.

Results indicate no statistical difference in the fecundity performance or fertility performance of any of the QRD 460 treatment groups when compared to the controls. The ER_{50} was >200 L as/ha for each time interval.

All validity criteria were met, i.e. mortality in the control group was ≤ 25%, fecundity in the control group was ≥ 2 and no more than five bugs laid zero eggs, fertility in the control group was ≥ 70% and the level of mortality in the reference item treatment was ≥ 40%.

Conclusion

The results of the study show that QRD 460 applied to glass surfaces caused statistically significant mortality to *Orius laevigatus*, when they were exposed one hour after spraying the test item. Mortality of juvenile bugs added 2, 4, 6 or 24 hours after spray treatment was not significantly different to untreated controls.

The LR_{50} of QRD 460 was >200.00 L as/ha for each time interval tested.

During the reproduction test exposure to QRD 460 did not result in a significant difference in reproductive capacity compared to the controls.

The ER_{50} was >200.00 L as/ha for each time interval tested and the NOEC for reproduction was 200.00 L as/ha.

([redacted]M, 2010c)

Report:	IIA 8.8.1.4/02 [redacted]M (2010d), Effects of QRD 460 on the plant dwelling insect, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) under Laboratory Conditions [redacted]. Final Report BT076/10, 10 December 2010

Guidelines

Schmuck *et al*. (2000). A laboratory test system for assessing effects of plant protection products on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera: Coccinellidae).

GLP: Yes.

Executive Summary

QRD460 (100% active ingredient), was sprayed on glass surfaces undiluted at 200 L as/ha and the insects (3 day old larva) were added at five different time intervals (1, 2, 4, 6 and 24 hours after spray treatment). Following pupation reproductive performance was tested: the number of fertile eggs was assessed for two weeks. The fertility test was conducted with the surviving females of the untreated control group and each test item group.

The results of the study show that QRD 460 applied to glass surfaces, caused no statistically significant mortality to *Coccinella septempunctata*, at any of the tested time intervals tested (insects added 1, 2, 4, 6 or 24 hours after spray treatment).

The LR_{50} of QRD 460 was >200.00 L as/ha for each time interval tested.

During the reproduction test exposure to QRD 460 did not result in a significant difference in reproductive capacity compared to the controls.

The NOEC for reproduction was 200.00 L as/ha.

Materials

Test Material:	QRD 460
Description:	Technical active ingredient for an EC insecticide formulation
Lot/Batch No.:	AQ421-13-2
Purity:	100%
Density:	Not reported
Stability:	Stable
Toxic standard	Perfekthion EC (393 g dimethoate/L), applied at a rate of 10 g product/ha in deionised water
Test rates:	200 L/ha (equivalent to 200 L a.s./ha)
Spray volume rate:	200 L/ha
Application method:	Potter tower

Test organisms

Species:	*Coccinella septempunctata* L. (Coleoptera: Coccinellidae)
Source:	[redacted]
Food:	Fresh aphids (*Aphis fabae* L.)
Age at test start:	3 day old larvae

Test design

Arenas: Glass container (5cm diameter, 3cm height), inner walls coated with talcum to prevent ladybird larvae and aphids climbing
Replication: 28
No. ladybirds/arena : 1

Environmental test conditions
Temperature: 21.33-27.33ºC
Humidity: 53.50-80.00% RH
Photoperiod: 16 h light and 8 h darkness
Duration of test: Approx 49 days

Study Design and Methods

Experimental dates: 22nd September – 10th November 2010.

Coccinella septempunctata three day old larvae were exposed to dried QRD460 residues on a glass surface until they completed ecdyses. QRD 460 was applied undiluted at 200 L as/ha and ladybird larvae were added 1, 2, 4, 6 or 24 hours later. The survival and development of larvae was recorded daily until metamorphosis was completed.

When more than 90% of the beetles were adults sex was determined and recorded. Hatched beetles from the untreated controls and each treatment were transferred to the reproduction unit. Assessment of reproductive performance was started one week after the appearance of the first egg batch in the untreated control groups. Egg counting was conducted for 14 days; the egg batches were stored under laboratory conditions until larval hatch. Hatching rate was recorded. Reduction in reproductive performance for each treatment group relative to the control was calculated.

Results and Discussion

Mortality and fecundity are summarised in Table IIA 8.8.1.4-2.

Table IIA 8.8.1.4-2: Effects of QRD 460 on mortality and fecundity of *Coccinella septempunctata*

Treatment rate – L a.s./ha	% mortality	Mean corrected % mortality	No. eggs/female/ assessment date	% hatching	No. fertile eggs/female/ assessment date	% effect on reproduction compared to control
0	14.29	na	9.92	87.82	8.81	Na
200 (1 hr AT)	28.57	16.66	13.88	87.83	11.04	-25.20
200 (2 hr AT)	7.14	0.00	12.60	87.83	8.95	-1.51
200 (4 hr AT)	21.43	8.33	10.63	73.67	8.25	6.40
200 (6 hr AT)	14.29	0.00	8.72	90.08	7.07	19.83
200 (24 hr AT)	16.67	2.78	9.54	88.84	7.90	10.34
Reference substance (3.65 g a.s./ha)	100.00	100.00	--	--	--	--

AT After treatment

Pre-imaginal mortality in the insects added 1, 2, 4, 6 and 24 hours after spray treatment was not statistically different from control mortality. The LR_{50} of QRD 460 was >200 L as/ha for each time interval tested.

Results indicate no statistical difference in the reproductive performance of any of the QRD 460 treatment groups compared to the control. The NOEC for reproduction was 200 L as/ha.

The study was considered valid because pre-imaginal mortality in the untreated control group didn't exceed 30%, the mean corrected mortality of the reference item group was > 40% and the mean number of fertile eggs/viable female/day in the untreated control was > 2.

Conclusion

QRD 460 applied to glass surfaces, caused no statistically significant mortality to *Coccinella septempunctata* at any of the tested time intervals tested (insects added 1, 2, 4, 6 or 24 hours after spray treatment).

The LR_{50} of QRD 460 was >200.00 L as/ha for each time interval tested.

During the reproduction test exposure to QRD 460 did not result in a significant difference in reproductive capacity compared to the controls.

The NOEC for reproduction was 200.00 L as/ha.

([redacted]M, 2010d)

IIA 8.8.2 Effects on non-target terrestrial arthropods in extended laboratory/semi field tests

None of the acute tests demonstrated any significant toxicity and so no further testing is warranted.

IIA 8.8.2.1 Parasitoid

None of the acute tests demonstrated any significant toxicity and so no further testing is warranted.

IIA 8.8.2.2 Predatory mites

None of the acute tests demonstrated any significant toxicity and so no further testing is warranted.

IIA 8.8.2.3 Ground dwelling predatory species (selected to be relevant to the intended uses of preparations)

None of the acute tests demonstrated any significant toxicity and so no further testing is warranted.

IIA 8.8.2.4 Foliage dwelling predatory species (selected to be relevant to the intended uses of preparations)

None of the acute tests demonstrated any significant toxicity and so no further testing is warranted.

IIA 8.8.2.5 Other terrestrial invertebrates

None of the acute tests demonstrated any significant toxicity and so no further testing is warranted.

IIA 8.9 Effects on earthworms

Theoretical calculations of the Koc's for the terpenoid blend QRD 460 components α-terpinene, ρ-cymene, d-limonene suggest a potential for accumulation in soil (see Section 5 Environmental Fate) and hence an acute earthworm study has been performed.

In the test results presented below, no toxicity was observed.

IIA 8.9.1 Acute toxicity to earthworms

Report:	IIA 8.9.1/01 [redacted] S (2011), QRD 452: A 14-day acute toxicity test with the earthworm *Eisenia fetida* (Oligocheata: Lumbricidae) [redacted] Study No 1145.004.630, 13 January 2011.

Guidelines

OECD Guideline for Testing of Chemicals No. 207 (1984): Earthworm Acute Toxicity Tests.

GLP: Yes.

Executive Summary

The acute toxicity of QRD 452 to the earthworm *Eisenia fetida* was determined. Earthworms were exposed to 62.5, 125, 250, 500 and 1000 test item/kg dry soil. No mortality was recorded in the test concentrations or the control group after 14 days. There was a statistically significant reduction in worm weight at the highest concentrations compared to the control.

The 14 day LC_{50} was >1000 mg ai/kg, and the NOEC (bodyweight) was 1000 mg ai/kg.

Materials

Test Material	QRD 452
Description:	Yellow liquid
Lot/Batch No.:	Lot # R-003
Purity:	17.41% total terpene (% by weight)
Stability:	Stable (expiry 7 Aug 2011)
Control	control and solvent (acetone) control
Test concentrations:	62.5, 125, 250, 500 and 1000 mg test item/kg dry soil
Toxic standard:	2-chloroacetamide in deionised water
Test organisms	
Species:	*Eisenia fetida* (Oligochaeta: Lumbricidae)
Source:	Culture source: [redacted]
Age and weight range of worms at test start:	Adult worms approximately 11 months old (300.2 to 592.8 mg weight range)
Food:	Pre-test: fed with horse manure, apple pomace and lucerne meal. No feeding during the test.
Test Design	
Test vessels:	1.5 L glass jars with loose fitting glass lids

Test substrate:	Artificial soil according to OECD Guideline # 207: The test substrate consisted of 70% industrial sand with more than 50% of particles between 80 and 200 µm ([redacted]), 20% kaolin clay with more than 30% kaolinite content ([redacted], Germany) and 10% sphagnum peat moss finely ground [redacted], Switzerland).
Replication:	4
No. of worms/vessel:	10

Environmental test conditions

Temperature:	19.8 to 20.6°C
pH of soil:	6.32 to 6.39 on Day 0 and 6.33 to 6.41 on Day 14
Water content of soil:	63.73% WHC, moisture content ranged from 38.68 to 41.26%.on Day 0 and 36.16 to 37.23% on Day 14. .
Photoperiod:	Continuous light (549 to 737 lux)
Duration of test:	14 days

Study Design and Methods

Experimental dates: 19 October to 02 November 2010

Earthworms were exposed to five concentrations of QRD 452: 62.5, 125, 250, 500 and 1000 mg test item/kg dry soil. These were compared to a control treatment and a toxic reference treatment of 2-chloroacetamide. The toxic reference test was carried out in a separate test run.

One day prior to experimental start, mature worms were isolated from the in-house culture in an unbiased fashion and placed on the artificial soil for acclimatization. On the day of experimental starting, the worms were individually weighed before they were placed as a group of ten on the treated soil of each of the four replicates.

After the mortality/health assessment on day 7, for which the soil of each beaker was emptied onto a tray to sort the earthworms, the soil was returned to the test vessel first and the worms were placed on the surface again after observations were performed.

At experimental completion, the mortality/health assessment was performed in the manner described above and the worms were individually re-weighed.

At test initiation, the stock solutions used for the application were measured by GC-FID. Recoveries were >90% and so results are based on nominal test concentrations.

Results and Discussion

On day 7 and after 14 days of exposure, no mortality was observed in all the treatments tested. Therefore, the 7 and 14-day NOEC and LC_{50} values for mortality were empirically estimated to be 1000 and > 1000 mg test item/kg dry soil, respectively.

At test start, mean earthworm weight was 437.4, 399.3, 414.6, 404.6, 418.5, 399.1 and 415.0 mg in the control, the solvent control and the 62.5, 125, 250, 500 and 1000 mg test item/kg dry soil treatments, respectively.

On day 14, mean earthworm weight was 385.0, 347.9, 371.2, 364.9, 386.3, 373.3 and 409.4 mg in the control, the solvent control and the 62.5, 125, 250, 500 and 1000 mg test item/kg dry soil treatments, respectively.

The weight differences between days 14 and 0 were -51.9, 43.5, -39.7, -32.3, -25.8 and -14.3 mg in the pooled controls, and the 62.5, 125, 250, 500 and 1000 mg test item/kg dry soil treatments, respectively. No statistically significant differences within the treatments were determined when compared to the pooled controls by using Kruskal-Wallis Test ($p > 0.05$).

For the reference standard test with 2-Chloroacetamide the 7 and 14-day LC_{50} values were within the range of 20 – 80 mg/kg dry soil stated in the ISO 11268-1 guideline.

Conclusions

After 14 days of exposure, no mortality was observed in all the treatments tested. Therefore, the 14-day NOEC and LC_{50} values for mortality were empirically estimated to be 1000 and > 1000 mg test item/kg dry soil, respectively. There was no significant effect on earthworm weight in any treatment i.e. NOEC of 1000 mg test item/kg dry soil.

([redacted]S, 2011)

IIA 8.9.2 Sublethal effects on earthworms

As there were no toxic effects of concern in the acute test, no further testing is warranted.

IIA 8.10 Impact on soil microbial activity

Due to its lack of intrinsic toxicity and as fugacity modelling (Section 5 Environmental Fate) suggests that the vast majority of QRD 460 is volatilised and dissipates into the air. Schocken (2011) describes the process by which soil microbes completely degrade p-cymene and d-limonene and due to the similarity between molecules, it is reasonable to expect the same processes for α-terpene. Therefore, microbial activity is unlikely to be affected by use of the plant protection product, not least of all as the terpene components are ubiquitous in nature.

IIA 8.10.1 Nitrogen transformation

Due to its lack of intrinsic toxicity and as fugacity modelling (Section 5 Environmental Fate) suggests that the majority of QRD 460 is volatilised and dissipates in the air, nitrogen transformation is unlikely to be affected by use of the plant protection product, not least of all as the terpene components are ubiquitous in nature.

IIA 8.10.2 Carbon mineralisation

Due to its lack of intrinsic toxicity and as fugacity modelling (Section 5 Environmental Fate) suggests that the majority of QRD 460 is volatilised and dissipates in the air, carbon mineralisation is unlikely to be affected by use of the plant protection product, not least of all as the terpene components are ubiquitous in nature.

IIA 8.10.3 Rates of recovery following treatment

Not relevant for QRD 460.

IIA 8.11 Effects on marine and estuarine organisms

No effects would be expected with QRD 460 as it is not soluble in water and primarily volatilises in air and dissipates rapidly.

IIA 8.11.1 Marine or estuarine organisms - acute toxicity lc50/ec50

No effects would be expected with QRD 460 as it is not soluble in water, primarily volatilises into air and dissipates rapidly.

IIA 8.11.2 Marine/estuarine fish - salinity challenge

This is not an EC data requirement.

IIA 8.12 Effects on terrestrial vascular plants

The mode of action of QRD 452 is as an insecticide which rapidly volatilises into the air and is not observed to have any significant interaction with plants. Combined with observations in all of the efficacy trials (details provided in the Biological Assessment Dossier, MIII Section 7) which indicate no effect on the quality of plants or plant products, it is not anticipated that application of QRD 452 will affect terrestrial vascular plants.

IIA 8.13 Effects on terrestrial vertebrates other than birds/wild mammal toxicity

None expected as QRD 460 contains α-terpinene, ρ-cymene, d- limonene which occur naturally in many plants that terrestrial vertebrates come in to contact with and consume in their normal lives. The plant protection use, therefore, is unlikely to contribute significantly to their natural exposure and raises no concern.

IIA 8.14 Effects on other non-target organisms (flora and fauna) believed to be at risk

Not applicable

IIA 8.14.1 Summary of all available data from preliminary tests used to assess biological activity and dose range finding, which may provide information on other non-target species (flora and fauna)

Not applicable

IIA 8.14.2 A critical assessment as to the relevance of the preliminary test data to potential impact on non-target species

Not applicable

IIA 8.15 Effects on biological methods for sewage treatment

As the plant protection use of QRD460 is unlikely to result in water contamination due to its rapid volatilisation, water insolubility, and low toxicity, its impact on sewage treatment is expected to be negligible.

IIA 8.16 Other/special studies

None relevant

IIA 8.16.1 Other/special laboratory studies

None relevant

IIA 8.16.2 Other/special field studies

None relevant

IIA 8.17 Summary and evaluation of points IIA 7 and IIA 8.1 to 8.16

Due to its chemical nature, Terpenoid blend (α-terpinene, ρ-cymene, d-limonene) QRD 460, disperses rapidly via volatilisation into air and leaves little to no residues (see Section 4 Metabolism and Residues). Equally it disperses rapidly in the environment into the air and then degrades rapidly (see Section 5 Environmental Fate), so any ecotoxicological exposure would be expected to be minimal.

The studies presented here demonstrate the expected lack of toxicity and that there are no ecotoxicological concerns with regard to the plant protection use of QRD 460 and its product QRD 452 presented here for registration.

As the levels of QRD 460 found on plants after application of the product QRD 452 have been shown to be minimal due to the rapid volatilisation of the active substance, the exposure of avian species to QRD 460 is not expected to be significant via the oral route or due to contact with treated foliage or fruits. Also, due to its rapid volatilisation from water, significant exposure is unlikely to occur to avians from drinking treated water. In one acute study on the Northern Bobwhite Quail, a low toxicity was demonstrated with the result of an LC_{50} > 2250 mg/kg. Mammalian studies from the Toxicology section also suggest a low level of toxicity to other species.

When it comes to water, it is clear from the physical/chemical properties of the terpene components in QRD 460 (α-terpinene, ρ-cymene, d- limonene) and their fugacity (see Section 5 Environmental Fate and Behaviour) that they are essentially insoluble in water and will volatilise into air and degrade rapidly whether applied directly to water or near water. Therefore, exposure to aquatic species is expected to be minimal. In a natural water degradation study, the three test items, α-terpinene, p-cymene, and d-limonene rapidly volatilized from the natural water test systems with DT_{50}s of 4.1, 11.2, and 4.1 hours and DT_{90}s of 13.7, 37.4 and 10.0 hours, respectively. Therefore, QRD 460 is also not available for exposure to aquatic organisms over long periods of time, so no chronic studies have been performed and no long term risk assessment is considered necessary. In one acute test on fathead minnow, a warm water fish species, the 24-, 48-, 72- and 96-hour LC_{50} values were empirically estimated to be > 1.17 mg test item/L and the 96-hour NOEC was determined by visual observation to be 1.17 mg test item/L, the highest level tested. This demonstrates a lack of significant toxicity to fish from the active substance QRD 460.

In the plant protection use of QRD 460, bioconcentration in fish would not be expected as it is essentially insoluble in water, volatilises readily, and breaks down rapidly in air after volatilisation.

From the plant protection use proposed, it is unlikely that there will be significant exposure to other aquatic organisms but an acute study on *Daphnia* has been performed. It should be noted that due to the high volatility and poor water solubility of QRD 460, it was not possible to attain the desired nominal concentrations. The problems encountered in this study due to the physical chemical properties of QRD 460 are also good reasons why exposure is expected to be so low in the practical usage of the plant protection product containing this active substance as to pose insignificant risk to aquatic organisms. The results of the Daphnia study indicate slight transient effects which were recovered from and the 24- and 48-hour EC_{50} values were empirically estimated to be >1.04 mg test item/L (mean measured concentration)

QRD 460 dissipates rapidly via volatilisation then breaks down readily in the air. From Section 5 Environmental Fate it has been shown that the three components, α-terpinene, ρ-cymene, d-limonene, are not persistent and dissipate in a matter of hours. As they are also highly insoluble, they do not remain in water very long after application as a plant protection product and, as such, their use is unlikely to result in any significant exposure of sediment dwelling organisms. A study on the acute toxicity to midge larvae (*Chironomus riparius*) under flow-through conditions was performed, and the 48-hour EC_{50} value was calculated to be 0.86 mg test item/L (95% confidence interval: 0.75 – 0.93 mg test item/L). The 48-hour NOEC was determined to be 0.360 mg test item/L.

As bees forage in many plants that naturally contain the terpene components of QRD 460, exposure to these compounds is likely to be a routine occurrence, e.g. from citrus blossoms. No acute oral study has been performed as it is more likely that bees would come into contact with the active substance during spraying or foraging on treated plants immediately after spraying. Consequently, two contact studies with honeybees were performed, one using QRD 420 and the other with the plant protection product formulation QRD 452. Both studies demonstrated a lack of toxicity at the highest levels tested.

There is a small possibility of exposure of non-target terrestrial arthropods and so a number of studies have been performed using the active substance on the aphid parasitoid *Aphidius rhopalosiphi*, the predatory mite,

*Typhlodromus py*ri, the predatory bug*, Orius laevigatus* and the plant dwelling insect, *Coccinella septempunctata L.* It should be noted that non-target terrestrial arthropods may well come into contact with the many plants that naturally contain the terpene components of QRD 460, and therefore exposure to these compounds is probably quite normal in the life of a non-target terrestrial arthropod. Therefore, exposure from the use of the plant protection product containing QRD 460 is unlikely to contribute significantly to any risk. In the four studies performed, no significant toxicity was observed and the LR_{50} of QRD 460 was > 200.00 L a.s./ha for each time interval tested which is significantly higher than the highest proposed filed rate. In all four studies performed, the ER_{50} was >200.00 L of a.s./ha at each time interval tested and the NOEC for reproduction was 200.00 L of a.s./ha.

Theoretical calculations of the Koc's for QRD 460 components α-terpinene, ρ-cymene, and d-limonene suggest a potential for accumulation in soil (see Section 5 Environmental Fate) and hence an acute earthworm study was performed. The three terpenes are highly volatile and dissipate rapidly into the air, a point supported by experimental evidence indicating that the likelihood of the product becoming bound in the soil compartment is rather small. This is supported with the fugacity modelling in Section 5 where almost all of QRD 460 is expected to partition to the air rather than soil or water. On this basis any risk to earthworms is unlikely to be significant. In the presented test, the 14 day LC_{50} was >1000 mg ai/kg, and the NOEC (bodyweight) was 1000 mg ai/kg, the highest concentration tested.

Due to the volatile nature of terpenoid blend (α-terpinene, ρ-cymene, d-limonene) QRD 460, and the fact that all three terpenes occur naturally and are ubiquitous it is reasonable to conclude that normal exposure presents no significant risk to humans, animals or the environment. Therefore, the plant protection use proposed here adds nothing of significance to the natural levels of exposure, it is believed that safety is confirmed and so no additional data is considered necessary.

In conclusion, strong evidence has been presented from Section 5 Environmental Fate to demonstrate that the terpenoid blend (α-terpinene, ρ-cymene, d-limonene) QRD 460 rapidly volatilises and dissipates predominantly into the air rather than the soil and water and one in the air, degrades rapidly. On this basis, it can already be proposed that exposure of organisms in the environment will not be significant from the plant protection use when compared to the naturally occurring terpenes and terpenoids in plants that are regularly released. However a number of acute tests have been performed and demonstrate that the toxicity to ecological species of concern from QRD 460 is low and not of concern. Most test studies have resulted with an endpoint at the highest test concentration or at the highest one possible to test as due to the volatilisation and insolubility, QRD 460 has shown low recoveries, as you would expect from a compound of this type. This is further evidence that supports the conclusion that exposure to the organisms in the environment will not be significant from the plant protection use proposed for t QRD 460 and Annex 1 listing is supported.

Overleaf is a table of the endpoints available, Table 8.17.

Table 8.17 Summary of Ecotoxicological Test Endpoints

Species	Test type	Test substance (a.s. or formulation)	Toxicological Endpoint	Test Guideline	Ref
Avian (IIA 8.1.1)					
Northern Bobwhite Quail	Acute oral, single dose	QRD 406 plant extract a.s.	LC_{50} > 2250 mg/kg	US-EPA, OPPTS Number 850.2100	[redacted]PM & [redacted]JB, 2007
Fish (IIA 8.2.1.2)					
Fathead minnow (*Pimephales promelas*)	Acute , 96 hr flow-through	QRD 460 a.s.	LC_{50} > 1.17 mg/L NOEC 1.17 mg /L	OECD 203	[redacted]M, 2011a
Invertebrates (IIA 8.3.1.1)					
Daphnia magna	Acute, 24 hr and 48 hr flow-through	QRD 460 a.s.	24- and 48-hour EC_{50} > 1.04 mg/L. 48-hour NOEC = 0.132 mg/L.	OECD 202	[redacted]M, 2011b
Invertebrates (IIA 8.3.2.1)					
Daphnia magna	Chronic, 21-day, flow-through	QRD 460 a.s.	21-day EC_{50} reproduction = 0.308 mg/L LOEC = 0.173 mg/L. NOEC = 0.214 mg/L.	OECD 211	[redacted]M, 2011c
Sediment Dwellers (IIA 8.5.1)					
Midge larvae (*Chironomus riparius*)	Acute, 48 hr flow-through	QRD 460 a.s.	48-hour EC_{50} = 0.86 mg/L 48-hour NOEC = 0.360 mg/L	OECD 202	[redacted]M, 2011d
Bees (IIA 8.7.2)					

Species	Test type	Test substance (a.s. or formulation)	Toxicological Endpoint	Test Guideline	Ref
01/ Honey Bee *Apis mellifera*	Acute contact, 48hr	QRD 420 a.s.+ canola oil	LD_{50} > 100 µg a.i./bee.	OECD 214	[redacted]R & [redacted]HO 2009a
02/ Honey Bee *Apis mellifera*	Acute contact, 48hr	QRD 452 16.75% EC formulation	LD_{50} > 100 µg a.i./bee.	OECD 214	[redacted]R & [redacted]HO 2009b
Non-target Terrestrial Arthropods (IIA 8.8.1.1)					
aphid parasitoid *Aphidius rhopalosiphi*	Acute contact, 24hr	QRD 460 a.s.	LR_{50} > 200.00 L a.s./ha ER_{50} > 200.00 L a.s./ha NOEC repro = 200.00 L a.s./ha	ESCORT	[redacted]M, 2010a
predatory mite, *Typhlodromus pyri*	Acute contact, 24hr	QRD 460 a.s.	LR_{50} > 200.00 L a.s./ha ER_{50} > 200.00 L a.s./ha NOEC repro 200.00 L a.s./ha	ESCORT	[redacted]M, 2010b
predatory bug, *Orius laevigatus*	Acute contact, 24hr	QRD 460 a.s.	LR_{50} >200.00 L a.s./ha ER_{50} > 200.00 L a.s./ha NOEC repro = 200.00 L a.s./ha	ESCORT	[redacted]M, 2010c
plant dwelling insect, *Coccinella septempunctata L.*	Acute contact, 24hr	QRD 460 a.s.	LR_{50} >200.00 L a.s./ha ER_{50} > 200.00 L a.s./ha NOEC repro = 200.00 L a.s./ha	ESCORT	[redacted]M, 2010d
Earthworms (IIA 8.9)					
Eisenia fetida	Acute, 14 day	QRD 452 16.75 EC formulation	LC_{50} > 1000 mg test item/kg dry soil NOEC = 1000 mg test item/kg dry soil.	OECD 207	[redacted]S 2011

9 JUSTIFIED PROPOSALS FOR THE CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE ACCORDING TO DIRECTIVE 67/548/EEC

Hazard symbol(s):

Indications of danger:	Xn	Harmful
	N	Dangerous for the environment
Risk phrases:	R22	Harmful if swallowed
	R43	May cause sensitisation by skin contact
	R51/53	Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
Safety phrases:	S16	Keep away from sources of ignition – No Smoking
	S24	Avoid contact with skin
	S29	Do not empty into drains
	S60	This material and its container must be disposed of as hazardous waste.
	S61	Avoid release to the environment. Refer to special instructions/safety data sheets.

Annex point/ reference number	Author(s)	Year	Title Sponsor/Source Test Facility, Report No GLP or GEP status (where relevant), Published or Not	Data Protection Claimed Y/N	Owner AQ = AgraQuest
IIA 8.1.1	[redacted] PM and [redacted] JB	2007	QRD-406: An acute oral toxicity study with the Northern Bobwhite, [redacted]. Report Number 489-114, 10 May 2007. GLP, Not Published	Y	AQ
IIA 8.2.1.2/01	[redacted] M	2011a	QRD 460: Acute toxicity to fathead minnow (Pimephales promelas) under flow-through conditions; [redacted] Study No. 1145.001.523, 11 February 2011. GLP, Not Published	Y	AQ
IIA 8.3.1.1/01	[redacted] M	2011b	QRD 460: Acute toxicity to Water Fleas (Daphnia magna) under flow-through conditions; [redacted] Study No. 1145.001.110, February 2011 GLP, Not Published	Y	AQ
IIA 8.3.2.1/01	[redacted] M	2011c	QRD 460: Chronic reproduction test with daphnids (Daphnia magna) under flow-through conditions, [redacted]. Study Number 1145.003.231, 13 May 2011 GLP, Not Published	Y	AQ
IIA 8.5.1/01	[redacted] M	2011d	Acute toxicity to midge larvae (Chironomus riparius) under flow-through conditions; [redacted] Study No. 1145.001.175, 11 February 2011. GLP, Not Published	Y	AQ
IIA 8.7.2/01	[redacted] JR and [redacted] HO	2009a	QRD 420: An Acute Contact Toxicity Study with the Honeybee, [redacted]. Project Number: 489-115, August 2009.	Y	AQ

			GLP, Not Published		
IIA 8.7.2/02	[redacted] JR and [redacted] HO	2009b	QRD 452: An Acute Contact Toxicity Study with the Honeybee, [redacted] Project Number: 489-116, August 2009 GLP, Not Published	Y	AQ
IIA 8.8.1.1/01	[redacted] M	2010a	Effects of QRD 460 on the aphid parasitoid Aphidius rhopalosiphi de stefani perez (Hymenoptera: Braconidae) under laboratory conditions. [redacted]: Final Report BT075/10 10 December 2010. GLP, Not Published	Y	AQ
IIA 8.8.1.2/01	[redacted] M	2010b	Effects of QRD 460 on the predatory mite, Typhlodromus pyri Scheuten (Acari: Phytoseiidae) under Laboratory Conditions, [redacted]. Final Report BT074/10 19 November 2010 GLP, Not Published	Y	AQ
IIA 8.8.1.4/01	[redacted] M	2010e	Effects of QRD 460 on the predatory bug, Orius laevigatus Fieber (Heteroptera: Anthocoridae) under Laboratory Conditions, [redacted]. Final Report BT077/10 10 December 2010 GLP, Not Published	Y	AQ
IIA 8.8.1.4/02	[redacted] M	2010d	Effects of QRD 460 on the plant dwelling insect, Coccinella septempunctata L. (Coleoptera: Coccinellidae) under Laboratory Conditions, [redacted] Final Report BT076/10 10 December 2010 GLP, Not Published	Y	AQ
IIA 8.9.1/01	[redacted] S	2011	QRD 452: A 14-day acute toxicity test with the earthworm Eisenia fetida (Oligochaeta: Lumbricidae), [redacted] Study No 1145.004.630 13 January 2011 GLP, Not Published	Y	AQ