Dossier According to Directive 91/414/EEC

REQUIEM EC (QRD 452)

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 MIII, Section 3

TOXICOLOGICAL STUDIES

Table of Contents

7 TOXICOLOGICAL STUDIES

Terpenoid Blend (α-terpinene, p-cymene, and d-limonene) QRD 460 is a new active substance developed by AgraQuest Inc. based originally on 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 7-1: EU Critical GAP for Requiem EC (QRD 452) 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 well known volatile nature of Terpenoid blend (α-terpinene, ρ-cymene, d- limonene) QRD 460, the fact that all three terpenoids occur naturally and are ubiquitous and normal exposure presents no significant risk to humans, animals or the environment, so the plant protection use proposed here is considered to add nothing of significance to the natural exposure, it is believed that safety is confirmed and so no additional data is considered necessary.

The components of the active substance have high vapor pressures and high Henry's Law Constants which means the active substance is highly volatile and evaporates quickly. In addition, it has been shown that the active substance does not persist in the environment. It has been demonstrated that following application of Requiem EC (QRD 452) as a foliar spray, the active substance constituents rapidly volatilize. Persistence on leaves is a matter of minutes and there are no detectable residues (See MII Section 4, Annex point IIA6.3). Because the active substance in QRD 452 dissipates so quickly from the sprayed plant surface, as well as the soil, water, and air, each application is in effect a single acute event. It is reasonable to conclude that even repeat applications may each be considered as single acute events rather than as chronic exposures. In addition, should exposure occur the active substance components have been shown to be rapidly metabolized and excreted in mammalian systems.

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% (w/w) 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.

IIIA 7.1 Acute toxicity

Acute toxicity studies have been conducted with QRD 416 and QRD 452, both EC formulations containing 16.75% by weight terpene constituents (α-terpinene, p-cymene, d-limonene) with identical co-formulants. Since the final composition of the two formulations is identical it is considered that results obtained with QRD 416 also apply to QRD 452. Full compositional information for both QRD 416 and QRD 452 is provided in Document J since this information is confidential.

QRD 452 is of low acute toxicity by the oral, dermal and inhalation routes. It is not irritating to the eyes or skin. Under the conditions of the Buehler QRD 452 was not a skin sensitiser, however, as the following discussion indicates, results from a LLNA test with QRD 452 were positive using this assay.

As previously indicated in MII section 3, the active substance QRD 460 did give a positive result in the LLNA test. However, it is important to consider that two other tests conducted with substantially similar active substances using the Buehler and Magnusson and Kligman methods were negative for sensitization. It seems unusual that a simple, previously tested, mixture of the three terpene constituents would result in a positive response only when the LLNA method is employed. d-Limonene has been classified as a weak sensitizer; however, this has been widely attributed to the presence of limonene oxidation products,[1] but neither α-terpene or ρ-cymene are classified as sensitizers. Literature indicates that QRD 460 does not contain sufficient d-limonene to trigger a strong sensitizing reaction, and internal investigations indicate that there are not significant amounts of limonene oxides in the test material, so the reason for the positive LLNA result is not easily explained.

The product, QRD 452, also gave a negative result for sensitization using the Buehler method. However, because the LLNA result for QRD 460 would override the Buehler result for QRD 452 in the hazard assessment, and a Calculation Rule would be applied, a LLNA test using QRD 452 was commissioned. The results of this test were positive; AgraQuest Inc. is investigating these new results.

In common with all toxicity tests, the LLNA is not 100% accurate, reports in the literature indicate it is approximately 90% reliable (similar to Magnusson and Kligman and Buehler), therefore, the possibility exists that the positive results reported for QRD 460 may not be indicative of the active substance's true biological nature. Potential false positives in the LLNA are not unprecedented. Other examples of materials implicated in this manner include: sodium lauryl sulfate, fatty acids such as oleic acid and linoleic acid, squalene, octinol, long-chain fatty acids, and non-ionic sugar lipid surfactants.[2]

Finally, real world experience with the plant extract-based and terpenoid blend active substances, as well as their respective formulated products, do not support the conclusions of the LLNA tests. The plant extract-based and terpenoid blend active substances have been manufactured for a number of years without a single report of dermal sensitization from manufacturing personnel. Similarly, the plant extract-based and QRD 452 plant protection products have been widely used (development trials and commercial use) in the USA with no reports of dermal sensitization or other adverse effects.

According to Commission Directives 67/548/EEC (as amended) and 1999/45/EEC and Regulation EC 1272/2008, the following classification or labelling is required.

[1] Christensson JB, Johansson S, Haqvall L, Jonsson C, Börje A, Karlberg AT. (2008) Limonene hydroperoxide analogues differ in allergenic activity. Contact Dermatitis, 59: 344–352

[2] [redacted] – personal communication to [redacted] (2011)

Table IIIA 7.1-1: Summary of Acute Toxicity

Study	Test substance	Result	Reference	Classification according to Dir 99/45/EC	Classification according to Reg. 1272/2008
Acute Oral	QRD 416	LD_{50} > 5000 mg/kg	[redacted]*J, 2008a*	None	None
Acute Dermal	QRD 416	LD_{50} > 2020 mg/kg	[redacted]*J, 2008b*	None	None
Acute Inhalation	QRD 452	LC_{50} > 5.19 mg/l	[redacted]*C, 2009*	None	None
Skin Irritation	QRD 452	Not irritant	[redacted]*J, 2009a*	None	None
Eye Irritation	QRD 452	Not Irritant	[redacted]*J, 2009b*	None	None
Skin sensitisation	QRD 452	Not a sensitiser	[redacted]*J, 2009c*	None	None
Skin sensitisation	QRD 452	Sensitiser	[redacted]*J, 2011*	R43 May cause sensitisation by skin contact	Cat. 1 H317 May cause an allergic skin reaction

IIIA 7.1.1 ACUTE ORAL TOXICITY

Report: IIIA 7.1.1**:** [redacted]J (2008a). QRD 416: Acute oral toxicity study (UDP) in rats. [redacted]. Laboratory Report No. 11783-08, 28 July 2008. Unpublished.

Guidelines

OECD 425 (2001): OPPTS 870.1100 (2002)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study

Executive Summary

The test substance, QRD 416, was evaluated for its acute oral toxicity potential in young adult female, Sprague-Dawley albino rats when administered as a gavage dose at 5000 mg/kg. The study was terminated following the stopping rules of this procedure. The test substance was dosed at a volume of 5.69 mL/kg. The rats were fasted overnight prior to dosing. They were assessed daily for the following 14 days for any signs of systemic toxicity and their body weights were recorded at intervals throughout the study. The animals were killed at the end of the study and were given a macroscopic examination *post mortem*.

There was no mortality. There were no clinical signs of toxicity and no effect on body weight gain. There were no abnormal findings at necropsy.

The acute oral LD_{50} of QRD 416 was estimated to be greater than 5000 mg/kg in female albino rats.

Materials:

Test Material:	QRD 416
Description:	Formulation; emulsifiable concentrate, pale yellow, low viscosity liquid with a 'woodsy' odour
Lot/Batch number:	T-Q-007 / 08-122GJ-02
Purity:	Confidential, see Document J
CAS#:	Not reported
Stability of test compound:	Reassay date: May 2010

Vehicle and/or positive control: None.

Test Animals:

Species	Rat
Strain	Sprague-Dawley albino
Age/weight at dosing	Young adult / 166-177 g (fasted weight)
Source	[redacted]
Housing	Individually in suspended, wire bottom, stainless steel cages.
Acclimatisation period	5 days
Diet	[redacted]™ Formulab #5008 *ad libitum*, except for approximately 16 hours prior to dosing.
Water	Municipal water *ad libitum*
Environmental conditions	Temperature: 19-22°C Humidity: 52-93% Air changes: 10-12 per hour Photoperiod: 12 hour light/12 dark cycle

Study Design and Methods:

In-life dates: Start: 20 May 2008 End: 5 June 2008

Animal assignment and treatment: In an acute oral toxicity study, a total of 3, young adult female Sprague-Dawley albino rats were given a single oral dose of 5000 mg/kg QRD 416 by gavage, following an overnight fast. The test substance was administered as received and was not diluted. An individual dose was calculated for each animal based on its fasted body weight and administered at a volume of 5.69 mL/kg.

Observations for mortality and clinical/behavioural signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14.

On Day 14 after dosing, each surviving animal was euthanized by an overdose of CO_2. All the animals were given a gross necropsy and all abnormalities were recorded.

Statistics: The LD_{50} value was estimated (limit dose, no mortality).

Results and Discussion

Mortality: There was no mortality.

Clinical observations: There were no clinical signs of toxicity.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at study termination revealed no observable abnormalities.

Conclusion

The acute oral LD_{50} of QRD 416 was estimated to be greater than 5000 mg/kg in female albino rats.

([redacted], 2008a)

IIIA 7.1.2 ACUTE PERCUTANEOUS (DERMAL) TOXICITY

Report:	IIIA 7.1.2: [redacted] (2008b). QRD 416: Acute dermal toxicity study in rats. [redacted]. Laboratory Report No. H1784-08, 9 July 2008. Unpublished.

Guidelines

OECD 402 (1987): OPPTS 870.1200 (1998)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study

Executive Summary

A group of five male and five female, young adult Sprague-Dawley rats were dermally exposed to 2020 mg (2.30 mL/kg) QRD 416/kg bodyweight. The test substance was tested as supplied. Test sites (not less than 10% of total body surface) were covered with an occlusive dressing for approximately 24 hours, after which the dressing was removed and the skin cleansed using clean water. The animals were assessed daily for the following 14 days for any signs of systemic toxicity. Observations for evidence of dermal irritation were made at approximately 60 minutes after removal of the wrappings and on days 4, 7, 11 and 14. Body weights were recorded just prior to dosing and on days 7 and 14. At the end of the study the animals were killed and subjected to a macroscopic examination *post mortem*.

No mortality occurred during the study. There were no clinical signs of toxicity or signs of dermal irritation at any time throughout the study. There was no effect on body weight gain. The gross necropsy conducted at termination of the study revealed no observable abnormalities.

The acute dermal LD_{50} of QRD 416 is greater than 2020 mg/kg in male and female rats (limit dose, no mortalities).

Materials:

Test Material:	QRD 416
Description:	Formulation: emulsifiable concentrate, pale yellow, low viscosity liquid with a 'woodsy' odour
Lot/Batch number:	08-122QJ-02
Purity:	Confidential, see Document J
CAS#:	Not reported
Stability of test compound:	Reassay date: May 2010

Vehicle and/or positive control: None

Test Animals:	
Species	Rat
Strain	Sprague-Dawley
Age/weight at dosing	Young adult / 269-294 g (males) and 177-197 g (females)
Source	[redacted]
Housing	Individually in suspended, wire bottom, stainless steel cages
Acclimatisation period	5 days
Diet	[redacted].TM Formulab #5008 ad libitum
Water	Municipal water ad libitum
Environmental conditions	Temperature: 19-22°C Humidity: 62-92 % Air changes: 10-12 per hour Photoperiod: 12 hour light/ 12 dark cycle

Study Design and Methods:

In-life dates: Start: 29 May 2008 End: 12 June 2008

Animal assignment and treatment: A group of five male and five female, young adult Sprague-Dawley rats were dermally exposed to 2020 mg QRD 416/kg bodyweight. The test substance was used undiluted, as supplied. Each animal was prepared on the day prior to treatment by clipping the dorsal surface of the trunk free of hair to expose not less than 10% of the total body surface area. Care was taken to avoid abrading the skin. Only those animals with exposure areas free of pre-existing skin irritation or defects were used for this study. All animals were treated with 2020 mg/kg (2.30 mL/kg) of undiluted test substance, evenly applied in a thin, uniform layer. The area of application was covered with a 2 x 4 inch surgical gauze patch secured with non-irritating adhesive tape. The trunk of each animal was then wrapped with vet wrap which was secured in place with non-irritating adhesive tape to prevent possible ingestion of the test substance. The application period was 24 hours. After 24 hours, the wrappings were removed. The test sites were gently washed with room temperature tap water and a clean cloth to remove as much residual test substance as possible.

Observations for mortality and clinical/behavioural signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. Observations for dermal irritation were made approximately 60 minutes after removal of the wrappings, and on Days 1, 4, 7, 11 and 14. On Day 14 after dosing, each animal was euthanized by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Statistics: The LD_{50} value was estimated (limit dose, no mortality).

Results and Discussion

Mortality: There was no mortality.

Clinical observations: All animals appeared normal for the duration of the study. There were no signs of dermal irritation at any observation during the study.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at study termination revealed no observable abnormalities.

Conclusion

The acute dermal LD_{50} of QRD 416 is greater than 2020 mg/kg in male and female rats.

([redacted] J, 2008b)

IIIA 7.1.3 ACUTE INHALATION TOXICITY TO RATS

Report:	IIIA 7.1.3 [redacted] C, (2009). QRD 452: Acute inhalation toxicity study in rats. [redacted]. Laboratory Report No. 12566-08, 4 March 2009. Unpublished.

Guidelines

OECD 403 (1981): OPPTS 870.1300 (1998)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study

Executive Summary

The test substance, QRD 452, was evaluated for its acute inhalation toxicity potential in young adult Sprague-Dawley albino rats. Five males and 5 females were exposed nose-only for 4 hours to an aerosol generated from the undiluted liquid test substance at a level of 5.19 mg/L. The concentration of the test substance in the exposure atmosphere was determined gravimetrically twice per hour and nominally at the end of the exposure. Following exposure, animals were retained for a 14 day observation period during which time they were observed at least once daily for clinical signs, and body weights were recorded just prior to the inhalation exposure, and on days 7 and 14. At the end of the study all animals were subjected to gross necropsy.

No animals died during the study. The only clinical sign was decreased activity. Body weight gain was unaffected by exposure, except in one female that lost weight between days 7 and 14. Gross necropsy revealed no observable abnormalities except discoloured liver or lungs in one male and two females.

The acute inhalation LC_{50} of QRD 452 is greater than 5.19 mg/L in male and female albino rats.

Materials:

Test Material: QRD 452

Description:	Technical grade; low viscosity, pale amber, aromatic liquid
Lot/Batch number:	R001
Purity:	25% technical grade a.i.
CAS#:	Not reported
Stability of test compound:	Not reported

Vehicle and/or positive control: None

Test Animals:

Species	Rat
Strain	Sprague-Dawley
Age/weight at dosing	Approximately 8 weeks old / 279-305 g (males); 163-196 g (females) at the start of exposure
Source	[redacted]
Housing	Individually in suspended, wire bottom, stainless steel cages
Acclimatisation period	5 days
Diet	[redacted].™ Formulab #5008 *ad libitum*, except during exposure
Water	Mains water *ad libitum* except during exposure.
Environmental conditions	Temperature: 20-23°C Humidity: 31-84%

Test Animals:

Air changes: 10-12 air changes/hour
Photoperiod: 12 hours light / 12 hours dark

Study Design and Methods:

In-life dates: Start: 16 January 2009 End: 30 January 2009

Exposure conditions: Trial assays were conducted to determine which method(s) of aerosolizing the test substance into the exposure chamber would produce an acceptable concentration and mass median aerodynamic diameter (MMAD).

Animal assignment and treatment: Five male and 5 female, young adult Sprague Dawley rats were exposed nose-only for 4 hours to an aerosol generated from QRD 452 at a level of 5.19 mg/L.

Prior to the start of the study they were examined to ensure that they were physically normal and exhibited normal activity. Observations for mortality and signs of pharmacologic and/or toxicological effects were made frequently on the day of exposure and then at least once daily thereafter for 14 days. Body weights were recorded just prior to exposure and on days 7 and 14. At the end of the scheduled period the animals were killed and examined *post mortem.*

Table 7.1.3-1: Mortality / animals treated

Exposure concentration mg/L	Mortality (Number dead / total)		
	Males	**Females**	**Combined**
5.19	0/5	0/5	0/10

Generation of the test atmosphere / chamber description: A 500 L nose-only stainless steel, dynamic flow inhalation chamber was used with polycarbonate tubes which were inserted into 10 designated individual ports. The aerosol was generated by pumping the test substance into a pressure operated air atomizer, then spraying the resulting aerosol directly into the exposure chamber. Air flow into the chamber was maintained through the use of a calibrated orifice plate at a rate of 23.76 air changes per hour. Air flow was recorded at 30 minute intervals during the exposure period, and was sufficient to ensure an oxygen content of at least 19% of the exposure atmosphere. Temperature and humidity were recorded at 30 minute intervals during the exposure period from a humidity/ temperature pen inserted in an unused port of the exposure chamber.

The animals were exposed to an aerosol generated from the undiluted liquid test substance for a period of four hours. The test substance was stirred continuously during exposure. When 99% concentration (t-99) was attained, the animals that were individually housed in polycarbonate exposure tubes were inserted into a 500 L stainless steel nose-only inhalation chamber for the specified exposure period. At the termination of the exposure period, the animals were returned to their stock laboratory cages.

Test atmosphere concentration: The concentration of test substance in the exposure atmosphere (taken from the breathing zone of the animals) was determined gravimetrically twice per hour and nominally at the end of the exposure. The gravimetric concentration was determined by passing a known volume of exposure air through a pre-weighed filter and dividing the amount of test substance deposited on the filter by the volume of air, which passed through the filter. The nominal concentration was determined by dividing the loss in weight of the test substance after the exposure by the total volume of air which passed through the chamber.

Particle size distribution: Particle size, taken from the breathing zone of the animals, was determined twice during the exposure, using a cascade impactor, at a rate of 8.7 L/minute for a duration of 30 seconds. The MMAD and particle size distributions are calculated from these data by a computer program utilizing probit analysis.

Table 7.1.3-2: Summary of acute study test atmosphere characteristics

Parameter		
Mean exposure concentration	5.19 mg/L	
Nominal concentration	34.3 mg/L	
Particle size MMAD; GSD	4.7, 4.4 µm; 5.6, 6.0 (at 1 hour and 3 hours into exposure respectively)	
Size range (µm)	% in size range	
	Run 1 (1 hour into exposure)	Run 2 (3 hours into exposure)
Particles 16.57 µm	0.00	1.71
Particles 9.89-16.57 µm	0.63	2.86
Particles 3.98-9.89 µm	2.53	2.29
Particles 2.40-3.98 µm	6.96	7.43
Particles 1.53-2.40 µm	14.56	13.71
Particles 0.85-1.53 µm	19.62	16.57
Particles 0.48-0.85 µm	29.11	28.57
Particles 0.28-0.28 µm	14.56	15.43
Particles 0.0-0.28 µm (backup filter)	12.03	11.43
Air atomizer setting: Sprayer air flow	26 L/min	
Air atomizer setting: Sample intake	3.7 mL/min	
Air flow rate	198 Lpm (n=9)	
Temperature	21°C (n=9)	
Humidity	20% (n=9)	

Statistics: In order to calculate a mean exposure, the Mean Value Theorem of Calculus was used to properly weight the concentration, since the concentrations could not be measured continuously. This method weights concentrations based on the time span of each concentration. A concentration can be calculated for each minute, which better represents the exposure concentration received by each animal.

The acute inhalation LC_{50} was estimated (limit test, no mortalities).

Results and Discussion

Mortality: There were no deaths during the exposure or observation periods.

Clinical observations: The only prominent in-life observation was decreased activity on days 0-2.

Body weight: Body weight gain was unaffected by exposure, except in one female that lost weight between days 7 and 14.

Necropsy: The gross necropsy revealed no abnormalities except discoloured liver or lungs in one male and two females.

Conclusion

The acute inhalation LC_{50} of QRD 452 is greater than 5.19 mg/L in male and female albino rats.

([redacted]C, 2009)

IIIA 7.1.4 SKIN IRRITATION

Report:	IIIA 7.1.4: [redacted]J, (2009a). QRD 452: Acute dermal irritation study in rabbits. [redacted]. Laboratory Report No. 12568-08, 4 February 2009. Unpublished.

Guidelines

OECD 404 (2002): OPPTS 870.2500 (1998)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study

Executive Summary

In a primary dermal irritation study, three, young adult (1 male and 2 female), New Zealand White rabbits were dermally exposed to 0.5 mL of QRD 452. The test substance was applied to a single intact skin site, approximately 2.5 cm x 2.5 cm on the dorsal trunk for 4 hours under a semi occlusive dressing. The application sites were observed for erythema and oedema and any other signs of skin irritation at 1, 24, 48 and 72 hours after bandage removal. Erythema and oedema were each scored on a 0-4 scale.

Neither erythema nor oedema was observed at any time throughout the study and no other signs of irritation were observed.

According to Commission Directives 67/548/EEC and 1999/45/EC and Regulation (EC) No 1272/2008 QRD 452 is non-irritating to skin and classification is not required.

Materials:

Test Material:	QRD 452
Description:	Technical grade; pale amber, low viscosity, aromatic liquid
Lot/Batch number:	R-001
Purity:	25% technical grade a.i.
CAS#:	Not reported
Stability of test compound:	Not reported

Vehicle and/or positive control: None

Test Animals:

Species	Rabbit
Strain	New Zealand White
Age/weight at dosing	Approximately 12 weeks / 2.3 kg male; 2.0-2.4 kg females
Source	[redacted]
Housing	Individually in suspended, wire-bottom, stainless steel cages
Acclimatisation period	5 days
Diet	Lab Rabbit Diet #5321 ([redacted]) 8 oz per day
Water	Municipal water *ad libitum*
Environmental conditions	Temperature: 19-22°C Humidity: 25-78% Air changes: 10-12 air changes/hour Photoperiod: 12-hour light/dark cycle

Study Design and Methods:

In-life dates: Start: 20 January 2009 End: 23 January 2009

Animal assignment and treatment: In a primary dermal irritation study, three, young adult (1 male and 2 females), New Zealand White rabbits were dermally exposed to 0.5 mL of QRD 452 (25% w/w a.i.).

The day before treatment, the dorsal area of the flank was clipped free of hair to expose an area at least 8 x 8 cm. Only those animals with exposure areas free of pre-existing skin irritation or defects were selected for testing. A single intact exposure site was selected as the test site while the contralateral intact site served as a control site.

On Day 0, 0.5 mL of undiluted test substance was applied to each test site and covered with a 4 ply surgical gauze patch measuring 2.5 x 2.5 cm. Each patch was secured in place with a strip of non-irritating adhesive tape. The entire trunk of each animal was loosely wrapped with a semi-permeable dressing (orthopedic stockinette) and secured on both edges with strips of tape to retard evaporation of volatile substances and to prevent possible ingestion of the test substance.

After four hours, the patches and wrappings were removed. The test sites were gently washed with room temperature tap water and a clean cloth to remove as much residual test substance as possible.

The animals were checked daily for signs of systemic toxicity and mortality. The test sites were observed for erythema and oedema formation, and any other dermal defects or irritation, at 1, 24, 48 and 72 hours after unwrap. Erythema and oedema were each scored on a 0-4 scale. For each animal, all of the erythema and oedema scores through 72 hours were added, and the sum was divided by 4 to obtain an individual irritation score. The primary irritation index was determined by calculating the mean of the irritation scores for all the animals and was used to obtain a rating for the test substance.

Results and Discussion

Neither erythema nor oedema was observed at any time throughout the study and no other signs of irritation were observed.

The Primary Irritation Index (PII) of 0.0 out of a possible 8.0 was obtained from the 1, 24, 48 and 72 hour observations.

Conclusion

Under the conditions of the study, QRD 452 is considered to be non-irritating to rabbit skin.

([redacted] J, 2009a)

IIIA 7.1.5 EYE IRRITATION

Report:	IIIA 7.1.5: [redacted] J, (2009b). QRD 452: Acute eye irritation study in rabbits. [redacted]. Laboratory Report No. 1256-08, 2 February 2009. Unpublished.

Guidelines

OECD 405 (2002): OPPTS 870.2400 (1998)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study

Executive Summary

In a primary eye irritation study, 0.1 mL of undiluted QRD 452 was placed into the conjunctival sac of the right eye of each of a group of 3 New Zealand White rabbits (1 male and 2 females). The grades of ocular reaction were recorded at 1, 24, 48 and 72 hours after treatment. The corneas of all treated eyes were examined immediately after the 24 hour observation with a fluorescein sodium ophthalmic solution. All treated eyes were washed with room temperature deionised water for one minute immediately after recording the 24-hour observation. Corneal opacity, iritis and conjunctival redness, chemosis and discharge were scored based on the Draize numerical scale. An average irritation score for each scheduled observation for all eyes was then determined, based on the number of animals tested. A maximum average irritation score was derived from the observation yielding the highest average irritation score. The maximum average irritation score was used to rate the test substance.

There were no positive effects exhibited in any eyes after treatment.

According to Commission Directives 67/548/EEC and 1999/45/EC and Regulation (EC) No 1272/2008 QRD 452 is non-irritating to skin and classification is not required.

Materials:

Test Material:	QRD 452
Description:	Technical grade; pale amber, low viscosity, aromatic liquid
Lot/Batch number:	R-001
Purity:	25% technical grade a.i.
CAS#:	Not reported
Stability of test compound:	Not reported

Vehicle and/or positive control: None

Test Animals:

Species	Rabbit
Strain	New Zealand White
Age/weight at dosing	Approximately 11-12 weeks / 2.70 kg (male), 3.125-3.350 kg (females)
Source	[redacted]
Housing	Individually in suspended, wire-bottom, stainless steel cages
Acclimatisation period	5 days
Diet	Lab Rabbit Diet #5321 ([redacted]) approximately 8 oz per day
Water	Municipal water ad libitum
Environmental conditions	Temperature: 15-21°C Humidity: 36-80% Air changes: 10-12 air changes/hour Photoperiod: 12-hour light/dark cycle

Study Design and Methods:

In-life dates: Start: 19 January 2009 End: 22 January 2009

Animal assignment and treatment: A primary eye irritation study was carried out using 3 New Zealand White rabbits (1 male and 2 females). Only animals without eye defects or irritation were selected for testing.

On Day 0, a dose of 0.1 mL of the undiluted test substance, QRD 452 (25% w/w a.i.) was placed into the conjunctival sac of the right eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance was dropped. The lids were gently held together for one second to prevent loss of material. The untreated left eyes served as comparative controls.

The treated eyes of all animals were examined without magnification under white room lighting, and (if needed), an additional source of white light or a handheld flashlight. The grades of ocular reaction were recorded at 1, 24, 48 and 72 hours after treatment. The corneas of all treated eyes were examined immediately after the 24 hour observation with a fluorescein sodium ophthalmic solution. An ocular transilluminator was used to enhance visualization of fluorescein staining. Any of the corneas which exhibited fluorescein staining at the 24 hour observation were re-examined with the fluorescein sodium ophthalmic solution at each consecutive observation until fluorescein staining of the cornea no longer occurred. All treated eyes were washed with room temperature deionised water for one minute immediately after recording the 24 hour observation.

Individual irritation scores for each animal at each scheduled observation were determined using a numerical grading scale similar to the Draize scale. An average irritation score for each scheduled observation for all eyes was then determined, based on the number of animals tested. A maximum average irritation score was derived from the observation yielding the highest average irritation score. The maximum average irritation score was used to rate the test substance. Any corneal involvement or iridial irritation with a score of 1 or more is considered positive. Any conjunctival irritation (redness or chemosis) with a score of 2 or more is considered positive.

Results and Discussion

Slight conjunctival redness was seen in one animal at the 1 hour reading. Chemosis and discharge was seen in two animals at the 1 hour reading. No abnormal findings were observed in the treated eye of any animal 24 hours after treatment.

The maximum average irritation score of 4.0, obtained at 1 hour after treatment, was used to rate QRD 452 minimally irritating. Fluorescein staining did not occur in any eyes.

Table 7.1.5-1: Eye irritation scores of QRD 452

Time	Cornea			Iris			Conjunctiva					
							Redness			Chemosis		
Animal number	**3304**	**3317**	**3319**	**3304**	**3317**	**3319**	**3304**	**3317**	**3319**	**3304**	**3317**	**3319**
after 1 hour	0	0	0	0	0	0	1 d	0	0d	1	0	1
after 24 hours	0	0	0	0	0	0	0	0	0	0	0	0
after 48 hours	0	0	0	0	0	0	0	0	0	0	0	0
after 72 hours	0	0	0	0	0	0	0	0	0	0	0	0
mean scores 24-72h	0	0	0	0	0	0	0	0	0	0	0	0

d – discharge

Conclusion

Based on the maximum average irritation score of 4.0, QRD 452 is rated minimally irritating. Since there were no positive effects during the study, the test substance is assigned to Toxicity Category IV. No irritation was observed in any eyes at 24 hours.

([redacted]J, 2009b)

IIIA 7.1.6 SKIN SENSITISATION

Report: IIA 7.1.6/01 [redacted] (2011). QRD 452 Skin sensitisation Local Lymph Node Assay in Mice. [redacted], USA. Laboratory Report No. 15083-11, 22 June 2011. Unpublished.

Guidelines

OPPTS 870.2600 (2003); OECD 429 (2010)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

Executive Summary

A skin sensitization study was conducted on 3 groups of 5 female mice to determine if test substance QRD 452 possesses a significant potential to cause skin sensitization. Five females were assigned to each of three groups, designated Groups I - III. The Test groups were treated with an appropriate dilution (25% or 50%) in acetone:olive oil vehicle, or undiluted test substance. Each animal received 25 µL to the dorsum of each ear. The animals were treated once daily for three days. After a two-day rest period, all animals were injected with tritiated methyl-thymidine in the tail vein. Five hours later, the animals were sacrificed, and the draining auricular lymph nodes removed and prepared for cell suspension and scintillation counting. A Vehicle Control group of five females was run concurrently, treated in the same manner with vehicle only instead of test substance or dilution. A Positive Control group of five females was also run concurrently, treated with 80% alpha-hexylcinnamaldehyde in acetone:olive oil.

The test substance produced a stimulation index of > 3 in all groups of Test animals, and is therefore considered a sensitizer (defined as producing a positive response).

Materials

Test Material:	QRD 452
Description:	Colourless liquid
Lot/Batch #:	# 00029201
Purity:	100%
Stability of test compound:	Not provided

Vehicle and/or positive control: Acetone:olive oil / alpha-hexylcinnamaldehyde

Test Animals:

Species	Mouse
Strain	CBA/J
Age/weight at dosing	Young adults / 17.5 – 23.2 g
Source	[redacted]
Housing	1-5 per cage
Acclimatisation period	At least 5 days
Diet	[redacted], Formalab #5008; *ad libitum*
Water	Municipal water supplied by an automatic system *ad libitum*
Environmental conditions	Temperature: 20-22°C Humidity: 37-92% Air changes: 10-12 changes/hour Photoperiod: Artificial, 12 hours light / 12 hours dark.

Study Design and Methods

In-life dates: Start: 11 May 2011 End: 16 May 2011

Healthy mice were released from quarantine prior to testing. Five females were selected for each of three Test groups (Groups I - III). On Days 1, 2 and 3, each Test animal in its group received an open application of 25 µL of an appropriate dilution (25% or 50%) of the test substance, or 100% test substance undiluted, to the dorsum of both ears. The Vehicle Control group (5 females) was treated in the same way as test animals, but with vehicle alone (acetone:olive oil) instead of test substance. The Positive Control group (5 females) was treated with 80% alpha-hexylcinnamaldehyde in acetone:olive oil. All Test and Control animals were given a two-day rest period on Days 4 and 5.

On Day 6 of the study, all Test and Control animals were injected in the tail vein with 250 µL of 0.01 M phosphate-buffered saline (PBS; Sigma, Lot 045K8210, Exp Jul 2015), pH 7.4, containing 20 µCi of [methyl, 1^1, 2^1-^{3}H] Thymidine (PerkinsElmer, Lot 201103, Exp Jan 2012). Five hours after the injection, the animals were sacrificed with an overdose of CO_2, the draining auricular lymph nodes were excised and pairs from each individual animal were processed.

A single cell suspension was prepared by gentle mechanical disintegration through 200 mesh stainless steel gauze. The cells were washed twice with an excess of PBS and precipitated with 5% trichloroacetic acid (TCA; Ricca Chemical, Lot 1009357, Exp Sep 2011) at 4° C for 18 hours. The pellets were resuspended in 1 mL of TCA and transferred to 10 mL of scintillation fluid. Incorporation of tritiated thymidine was measured by liquid scintillation counting as disintegrations per minute (DPM) from the paired lymph nodes of each animal, and mean DPM/animal was calculated for each group.

Results and Discussion

One Test Group 2 animal failed to gain weight and three Test Group 3 animals lost weight during the study; one Vehicle Control and all Positive Controls also lost weight. Signs of clinical toxicity are presented in Table 2. All animals appeared normal for the duration of the study.

Individual DPM counts are presented in Table IIA 5.2.6-1. The Stimulation Index (SI) or Test/Vehicle Control Ratio

derived for each Test group based on the group mean DPM is as follows:

Table IIA 5.2.6-1: Radiolabel incorporation into lymph-nodes of mice treated with QRD 452

Animal Group	Test Substance Concentration	Average Count per Mouse	No. of Mice in Group	Test/Vehicle Control Ratio
Vehicle Control	NA	432	5	NA
Test Group I	25%	2569	5	6.0
Test Group II	50%	6929	5	16.0
Test Group III	100%	7965	5	18.4
Positive Control	NA	13014	5	30.1*

NA – Not Applicable
* - Positive control used to confirm animal sensitisation and validate procedures.

Conclusions

QRD 452 produced a stimulation index of ≥ 3 in all groups of test animal, and is therefore considered a sensitiser (defined as producing a positive response).

([redacted]J, 2011)

Report: IIIA 7.1.6/02 [redacted], (2009c). QRD 452: Skin sensitization study in guinea pigs/ [redacted]. Laboratory Report No. 12569-08. Issue date 15 April 2009. Unpublished.

Guidelines

OECD 406 (1992): OPPTS 870.2600 (1998)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study

Executive Summary

A skin sensitization study, based on the method described by Ritz and Buehler, 1980**,** was conducted on 15 male and 15 female short-haired Hartley-Albino guinea pigs to determine if test substance QRD 452 produced a sensitising reaction. Animals were assigned to each of two groups, designated Groups I and II. Group I animals (5 per sex) remained untreated during the induction phase of the study and served as a naive control group. Group II animals (10 per sex) the test group, were treated with 0.4 mL of undiluted test substance (selected from previous screening). The animals were treated once weekly for three weeks, i.e. a total of three treatments. After a two-week rest period, all animals (Groups I and II) were challenged at a virgin test site with an application of 0.4 mL of undiluted test substance.

The sensitivity of guinea pigs to a positive control material, 85% alpha-hexylcinnamaldehyde, was confirmed.

QRD 452 produced no irritation in the test animals (Group II) or the naive control animals (Group I) after the challenge treatment, and therefore did not elicit a sensitizing reaction in guinea pigs.

QRD 452 was not a skin sensitiser under the conditions of the test.

Materials:

Test Material:	QRD 452
Description:	Technical grade; pale amber, low viscosity, aromatic liquid
Lot/Batch number:	R-001
Purity:	25% technical grade a.i.
CAS#:	Not reported
Stability of test compound:	Not reported

Vehicle and/or positive control: None / positive control was 85% alpha-hexylcinnamaldehyde.

Test Animals:	
Species	Guinea pig
Strain	Hartley-Albino
Age/weight at dosing	5-6 weeks / 353-464 g males and 340-407 g females
Source	[redacted]
Housing	1-4 per cage (sexes separately) in suspended, wire bottom, stainless steel cages
Acclimatisation period	5 days
Diet	[redacted] ad libitum
Water	Municipal water ad libitum
Environmental conditions	Temperature: 15-24°C Humidity: 24-98% Air changes: 10-12 per hour Photoperiod: 12-hour light/dark cycle

Study Design and Methods:

In-life dates: Start: 25 February 2009 End: 27 March 2009 (main study)

Start: 5 June 2008 End: 5 July 2008 (positive control study)

Animal assignment and treatment: The sensitisation potential of the test substance was assessed using a method based on that described by Ritz and Buehler. Two main procedures were involved; (a) the potential induction of an immune response; (b) a challenge of that response. Young adult, Hartley albino guinea pigs were assigned to each of two groups, designated Groups I and II. Group I animals (5 per sex) remained untreated during the induction phase of the study and served as a naive control group. Group II animals (10 per sex), the test group, were treated with 0.4 mL of undiluted test substance. A preliminary irritation test was carried out to determine the highest non-irritating concentration (HNIC) of the test substance prior to the challenge dose. The HNIC selected for the challenge phase was 100%.

On the day prior to each treatment, the animals were prepared by clipping the back of the trunk free of hair to expose a longitudinal area at least 8 x 10 cm on each animal. Individual body weights were recorded on Days 0 and 31.

Induction: For each induction treatment, Group II animals were treated with 0.4 mL undiluted test substance beneath a 4 ply, 2.5 x 2.5 cm surgical gauze patch on the left front quadrant of the exposure and secured with a strip of non-irritating adhesive tape. A strip of clear polyethylene film was placed over the patch and securely taped. Each animal was then placed in a restrainer for approximately six hours. At the end of the exposure period, the animals were removed from the restrainers, the wrappings and patches were removed, and the animals were returned to their cages. Group II animals were treated once weekly for three weeks, on days 1, 8 and 15. The same treatment regimen and test site location was used for all three induction treatments. Group I animals remained untreated during the induction phase of the study.

Observations for skin reactions at each test site were made approximately 24 hours after each treatment and approximately 48 hours after the first induction treatment. Erythema was scored on a 0-3 scale.

Challenge: After a two week rest period, all animals (Groups I and II) were each challenged at a virgin test site with an application of 0.4 mL of undiluted QRD 452. The challenge treatment was on Day 29. The dose was applied in a manner identical to the induction treatments, except the test site was placed laterally on the right rear quadrant of the exposure area.

Observations for skin reactions at each test site were made approximately 24 and 48 hours after challenge. Erythema was scored on a 0-3 scale.

An average score for each time period was obtained by adding all of the scores for each time period and dividing by the number of test sites scored for that time period. The test substance is considered a sensitizer if the mean irritation scores, the total number of animals with scores, and/or the total number of scores for the virgin test site in the test group after the challenge treatment are appreciably greater than those for the naive challenge group.

Positive Controls: The sensitivity of guinea pigs to a positive control material (alpha-hexylcinnamaldehyde, 85%) was confirmed in this laboratory. Induction and challenge applications used the neat test substance.

Results and Discussion

Mortality / Clinical observations: All animals survived till the end of the study. No abnormal behaviour or clinical signs were detected.

Body weights: There were no treatment-related effects on body weight during the study.

Induction reactions and duration: There were no signs of irritation.

Challenge reactions and duration: There were no signs of irritation.

Positive control: Faint to strong erythema was seen in 10/10 animals twenty-four hours after the end of the challenge exposure and very faint to faint erythema was present in six animals at the 48 hour reading. A mean score of 1.2 for the test group after challenge treatment, when compared with the naive control group mean score of 0.1, confirmed the sensitivity of the strain of animals used and the reliability of the experimental technique.

Table 7.1.6-1: Buehler test: Number of animals with positive signs of allergic skin reactions following challenge

	Test flank	
	Challenge at 100%	
Scored after:	**24 hours**	**48 hours**
Main test – test group	0/20	0/20
Main test – negative vehicle control	0/10	0/10
	Challenge at 100%	
	24 hours	**48 hours**
Positive control – test group	10/10	6/10
Positive control – vehicle control	3/10	0/10

Conclusion

QRD 452 was not a skin sensitiser under the conditions of the test.

Reference

H.L. Ritz and E.V. Buehler, "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests," Current Concepts in Cutaneous Toxicity, p. 25-42, Academic Press, NY, 1980)

([redacted]J, 2009c)

IIIA 7.1.7 SUPPLEMENTARY STUDIES FOR COMBINATIONS OF PLANT PROTECTION PRODUCTS

Not relevant, QRD 452 will not be recommended for use in tank-mixture with other plant protection products.

IIIA 7.2 Short-term toxicity studies

This is not an EC data requirement.

IIIA 7.3 Operator exposure

QRD 452 is an emulsifiable concentrate (EC) formulation containing 152.3 g/L QRD 460. It is intended for insect control in glasshouse crops (tomato, pepper, melon and cucumber). Field uses are also proposed for melon and cucumber. The recommended use conditions are summarised in the following table.

Table IIIA 7.3-1: Crops and use pattern proposed for QRD 452

Situation	Crop	Recommended maximum use rates (kg a.s./ha)	Spray volumes (l/ha)	Maximum recommended in-use concentration (g a.s./hl)	Application techniques
Glasshouse (high and low crops)	Tomato, Pepper, Cucumber, Melon	1.523	400 - 1000	381	knapsack tractor mounted boom
Field (low crops)	Melon, Cucumber	1.523	400 - 1000	381	knapsack tractor mounted boom

The maximum proposed label rate is 10 L product/ha (1.523 kg a.s./ha) however for most use scenarios the typical rate applied will be lower. QRD 452 will be sold in 1 and 2.5 US gallon containers (approximately 3.75 and 9.4 L) with 50 – 63 mm closures. For the following assessment 5 L containers are used for both hand held and tractor based applications as the closest representative of the smallest pack.

QRD 452 is of low acute toxicity by the oral, dermal and inhalation route. It is considered to be not irritating to skin and eyes. QRD 452 is considered to be a skin sensitiser under the conditions of LLNA but it has not shown a potential for skin sensitisation under the conditions of the Buehler test.

All three terpene components of QRD 452 (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. 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. The additional plant protection use proposed here adds nothing of significance to the natural exposure and hence is not expected to present significant risk to humans, animals or the environment.

For glasshouse applications the Dutch model[1] for calculating operator exposure during mixing/loading and application for upward and downward spraying was used.

Operator exposure estimates for outdoor uses are calculated using both the German model[2] and the UK POEM[3]. For tractor applications calculations using both the UK POEM and German model are presented. Since only low crops are to be treated in the field just the UK POEM for hand held applications has been used since the German model only contains data for upwards spraying using hand held equipment.

Since QRD 452 is volatile it is possible that the respiratory exposure to operators is underestimated by these models. Therefore estimates of respiratory exposure of the operator for applications in glasshouses as a result of volatilisation are presented as the worst case.

Data used for the calculations

Area treated per day:

Tractor applications outdoor:
20 ha for the calculations using the German Model;
50 ha for the calculations using the UK POEM

Hand held applications indoor and outdoor:
1 ha (or maximum 400 L/day, UK POEM only)

Application rates (maximum); 1.523 kg a.s./ha

Spray volume (minimum): (for UK POEM only) 400 L/ha

Package size and type: (for UK POEM only) 5 L

Standard operator body weight: 70 kg for Dutch and German models
60 kg for UK POEM

Absorption data

Dermal absorption has not been determined for QRD 452. QRD 452 has been shown to be of low acute toxicity by the dermal route (LD_{50} > 2020 mg/kg). Worst case values of 100% dermal absorption will therefore be used for the assessment.

Acceptable Operator Exposure Level:

The full set of studies usually considered relevant for the derivation of an AOEL is not available for QRD 460. Whilst no data are available on QRD 460 to address short or long term toxicity, AgraQuest Inc. believe use of products containing QRD 460 will not result in repeated human exposure to QRD 460 by the oral, inhalation or dermal routes (see section IIA 5.3 in the QRD 460 dossier for full details). Furthermore the acute data on the active ingredient, formulation and short/long term toxicity data on the components of QRD460 generally indicate low toxicity. No AOEL is proposed for QRD 460.

The components of QRD 460 (α-terpinene, ρ-cymene, and d-limonene) are naturally occurring in a multitude of fruits, vegetables, herbs, spices, and other foods and beverages, including coffee, tea, alcoholic beverages, baked and fried potatoes, bread and cheese. Further information on terpene levels in food stuffs is presented in Document MII Section 4 of the QRD 460 dossier. In addition to the natural occurrence, the active ingredient components of

[1] Van Golstein Brouwers YGC, Marquart J and Van Hemmen JJ (1996). Assessment of occupational exposure to pesticides in agriculture. Part IV. Protocol for the use of generic exposure data. TNO Nutrition and Food Research Institute, The Netherlands, TNO Report V96.120

[2] Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protections); Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirschaft, Berlin-Dahlem, n° 277, 1992

[3] Scientific Subcommittee on Pesticides and British Agrochemicals Joint Medical Panel., Estimation of Exposure and Absorption of Pesticides by Spray Operators (UK MAFF) 1986 and the Predictive Operator Exposure Model (POEM) (UK MAFF) 1992.

QRD 460 are permitted for use as food additives in the US and Europe, and as fragrance additives in cosmetics. Although the levels are relatively low, the general public is further exposed to these components through ingestion, dermal contact, and inhalation on a daily basis. According to a 2005 World Health Organization (WHO) report on food additives, the per capita daily consumption of the three main components as food additives in the US and Europe, respectively, are as follows: d-limonene, 12.76 mg and 39.307 mg; p-cymene, 0.472 mg and 1.085 mg; α-terpinene, 0.093 mg and 0.032 mg.

The Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) report Maximised Survey-derived Daily Intakes (MSDI).

$$\text{MSDI (µg/capita/day)} = \frac{\text{Annual production (kg)} \times 10^9 \text{ (µg/kg)}}{\text{Consumers} \times \text{survey response rate} \times 365 \text{ (days)}}$$

Notes:

Annual production volume in one year in Europe

Consumers: estimated to be 10% of the total European population (=32,000,000)

Survey response rate: correction made to take account that data provided by industry may be incomplete (= 0.6 in Europe)

European MSDI values for p-cymene of 926 µg/capita/day[4], α-terpinene of 27 µg/capita/day[5] and d-limonene of 33542 µg/capita/day are reported. All were considered of no safety concern at the estimated levels of intake.

Furthermore JECFA[6] has established an acceptable daily intake (ADI) "not specified" for d-limonene. This reflects the lack of health concern associated with dietary exposure to d-Limonene and means there is no intake level which is considered to be harmful over a life-time.

Since an AOEL can't be derived and it is not possible to quantify dietary exposure to the terpenes occurring naturally in food, instead exposure for operators, workers, bystanders following the proposed uses of QRD 452 will be compared with background daily exposure to the terpene components via dietary intake as a result of their use as food additives. The total MSDI for the three terpene components is 34495 µg/capita/day (34.5 mg/day). This can be considered worst case as no account is made for consumption resulting from natural occurrence of the terpenes in food.

IIIA 7.3.1 ESTIMATION OF OPERATOR EXPOSURE ASSUMING PERSONAL PROTECTIVE EQUIPMENT IS NOT USED

Operator exposure values have been calculated according to the exposure models and model parameters described above. For details of the calculations refer to Appendix 1.

The model calculation of the estimated operator exposure assuming that PPE is not used, considers the following clothing:

UK-POEM	No PPE	Long sleeved shirt, long trousers ("permeable") and no gloves.
German Model	No PPE	Short sleeved shirt and shorts, no gloves
Dutch Model	No PPE	Not defined

[4] Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to Flavouring Group Evaluation 18 (FGE.18): Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical group 6 (2006). The EFSA Journal 331, 1-77.

5 Flavouring Group Evaluation 25, (FGE.25)[1] - Aliphatic and aromatic hydrocarbons from chemical group 31 - Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (2008). The EFSA Journal 918, 1-109.

[6] http://apps.who.int/ipsc/database/evaluations/chemical.aspx?chemID=558

Results of the model calculations for QRD 452 and comparison with the total MSDI

The results of the operator exposure estimates for the proposed uses at the maximum application rate of 1.523 kg a.s./ha and the percent account of the combined MSDI are summarized in the following table.

Table IIIA 7.3.1-1: Estimated operator exposure values and % of MSDI – without PPE

	Total systemic exposure (mg/day)	% of MSDI (34.5 mg/day)
Glasshouse Applications		
Dutch Model	307.93	893
Field Applications		
UK POEM – Tractor Hydraulic	310.73	901
UK POEM – Hand-held (downwards application)	429.94	1246
German Model – Tractor Field Crop	123.33	358

According to the applied models the estimated operator exposures account for more than 100% of the total MSDI for the terpenes. Operator exposure to the terpene components in QRD 460 is therefore potentially greater than that via dietary intake in the absence of PPE.

IIIA 7.3.2 ESTIMATION OF OPERATOR EXPOSURE ASSUMING PERSONAL PROTECTIVE EQUIPMENT IS USED

Operator exposure levels have been calculated using the models and parameters described above.

The model calculations of the estimated operator exposure assuming PPE is used consider the following clothing:

UK-POEM	PPE	Tractor Application Long sleeved shirt, long trousers ("permeable") and protective gloves during mixing/loading and application Hand held Application Long sleeved shirt, long trousers ("permeable") and protective gloves during mixing/loading and gloves plus impermeable coveralls during application
German Model	PPE	Coverall, hat or cap, solid shoes and protective gloves during mixing and loading
Dutch Model	PPE	Model does not specify

Results of the model calculations for QRD 452 and comparison with the total MSDI

The results of the operator exposure estimates and their percent account of the total MSDI are summarized in the following table.

Table IIIA 7.3.2-1: Estimated operator exposure values and % of MSDI – with PPE

	Total systemic exposure (mg/day)	% of MSDI (34.5 mg/day)
Glasshouse Applications		
Dutch Model	30.79	89
Field Applications		
UK POEM – Tractor Hydraulic	40.02	116
UK POEM – Hand-held (downwards application)	75.96	220
German Model – Tractor Field Crop	4.35	13

For applications in glasshouses the Dutch model indicates that for applications at rates up to and including 1.523 kg a.s./ha acceptable risk to operators is demonstrated with the use of appropriate protective clothing.

Based on the German model acceptable risk to operators can be concluded with the use of appropriate protective clothing.

Calculations using the UK POEM indicate that risk for field applications at 1.523 kg a.s./ha when applied using a tractor mounted hydraulic boom will probably be acceptable since exposure is only slightly greater than the total MSDI for the three terpenes. For hand held applications a potential risk for operators is indicated. The calculations so far have assumed an application volume of 400 L/ha however optimum efficacy of QRD 452 is achieved when the product is applied in larger volumes of water and hence these will be more typical of the general use of QRD 452. The UK POEM scenarios have therefore been re-run using the maximum water volume of 1000 L/ha.

Table IIIA 7.3.2-2: Estimated operator exposure values and % of MSDI based on application volumes of 1000 L/ha and with PPE

	Total systemic exposure (mg/day)	% of MSDI (34.5 mg/day)
UK POEM – Tractor Hydraulic	25.15	73
UK POEM – Hand-held (downwards application)	32.85	95

For applications at volumes of 1000 L/ha and using appropriate PPE acceptable risk to operators is demonstrated using UK POEM.

Respiratory Exposure due to Volatilisation

All three terpenoid components of the active substance QRD 460 are extremely volatile by nature and QRD 460 will degrade rapidly in air to form smaller, naturally occurring molecules. Please refer to point IIA 7.10 of the QRD 460 dossier for full details on the rate and route of degradation in air.

Since QRD 452 is volatile, it may be that the respiratory exposure to operators is underestimated by the standard models. For field applications it is considered that the rapid dissipation and dilution into the environment will mean that inhalation is not a significant exposure route for operators. For the assessment of inhalation exposure to operators in greenhouses the predicted environmental concentration PEC_{AIR} has been calculated according to SANCO/10553/2006 rev. 2[7].

[7] SANCO/10553/2006 Rev 2, Pesticides in Air: Considerations for Exposure Assessment, Report prepared by the FOCUS Working Group on Pesticides in Air, June 2008

The calculation of PEC_{AIR} uses the maximum application rate of 1.523 kg a.s./ha and assumes a typical EU glasshouse with an area of 256 m^2 and a total volume of 901 m^3. It is assumed all three active components of the plant protection product (α-terpinene, p-cymene, and d-limonene) volatilize immediately and completely after application and that the glasshouse ventilation rate is 33%/hour. On this basis PEC_{AIR} is estimated to be 0.043 mg/L (43 mg/m^3).

It should be noted that all evidence from modelling, the literature and anecdotal evidence suggests that none of the terpenoid constituents of QRD 460 persist in the air and all are rapidly broken down. This means that the PEC_{AIR} value as calculated is worst case and any exposure is very short lived. However it is not possible to quantify this precisely from the information available.

Respiratory exposure for the operator over a given period of time can be calculated using concentrations in air over the relevant time period and a standard respiration rate of 1.25 m^3/h. However since reliable estimates of QRD 460 air concentrations over time are not available it is not appropriate to perform the calculations.

Since it cannot be excluded that operator exposures in glasshouses will not be greater than those from naturally occurring sources of the terpene components of QRD 460 it is recommended operators may want to consider use of appropriate respiratory equipment during mixing/loading and application of products containing QRD 460.

Summary

Whilst the models do not demonstrate acceptable risk for all use scenarios when taking the following additional factors into account it is considered reasonable to conclude that use of QRD 452 will not pose an unacceptable risk to operators when applied according to the proposed GAP and with the use of appropriate PPE and hygiene measures.

- The plant protection product QRD 452, the active substance QRD 460 and its constituents (α-terpinene, p-cymene, and d-limonene) have all been shown to be of generally low acute toxicity by the oral, dermal and inhalation routes. The available data on α-terpinene, p-cymene, and d-limonene indicate the components of QRD 460 are readily metabolised to materials which are excreted within 48 hours. As a result operator exposure via oral, dermal and inhalation routes is not expected to result in systemic toxicity.
- Use of products containing QRD 460 will not result in repeated human exposure to QRD 460 by the oral, inhalation or dermal routes (see point IIA 5.3 of the dossier on QRD 460 for full details).
- The relatively high application volumes (400 – 1000 L/ha) result in lower 'in-use' concentrations of plant protection product and hence lower exposure potential during application
- The components of QRD 460 (α-terpinene, p-cymene, and d-limonene) are naturally occurring in a multitude of fruits, vegetables, herbs, spices, and other foods and beverages, including coffee, tea, alcoholic beverages, baked and fried potatoes, bread and cheese. In addition to the natural occurrence, the active ingredient components of QRD 460 are permitted for use as food additives in the US and Europe, and as fragrance additives in cosmetics. Although the levels are relatively low, the general public is exposed to these components through ingestion, dermal contact, and inhalation on a daily basis. According to a 2005 World Health Organization (WHO) report on food additives, the per capita daily consumption of the three main components as food additives in the US and Europe, respectively, are as follows: d-limonene, 12.76 mg and 39.307 mg; p-cymene, 0.472 mg and 1.085 mg; α-terpinene, 0.093 mg and 0.032 mg. The Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) report Maximised Survey-derived Daily Intakes (MSDI). European MSDI values for p-cymene of 926 µg/capita/day[8], α-terpinene of 27 µg/capita/day[9] and d-limonene of 33542 µg/capita/day[21] are reported. All

[8] Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to Flavouring Group Evaluation 18 (FGE.18): Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical group 6 (2006). The EFSA Journal 331, 1-77.

9 Flavouring Group Evaluation 25, (FGE.25)[1] - Aliphatic and aromatic hydrocarbons from chemical group 31 - Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (2008). The EFSA Journal 918, 1-109.

were considered of no safety concern at the estimated levels of intake. Furthermore the ADI established by JECFA for d-limonene is 'not specified'.

- It is relevant to note that the WHO and EFSA reports referenced above only take into account the average daily intake of the three terpenes based on their use as food additives, flavourings, processing aids and materials in contact with food..

  These reports do not estimate the average daily intake of the three terpenes from the many foods that naturally contain these substances. As previously noted, they occur naturally in a multitude of fruits (especially citrus), vegetables, herbs, spices and other foods and beverages, including coffee, tea, alcoholic beverages, baked and fried potatoes, bread and cheese. A recent survey (Hakim *et.*al 2002) in the US assessing daily intake of d-limonene found the mean intake by consumers from citrus juices alone was 13.0 to 13.2 mg/day. See Schocken (2011) MII, section 4, point 6.2 for examples of foods where levels of the three terpenes have been quantified.

  In addition to exposure from additives and through foods, the general public is exposed to the three tepenes (d-limonene, terpinene, p-cymene), through dermal contact (cosmetics, household products), and inhalation (released by plants and household products) on a daily basis. WHO (IPCS: Concise International Chemical Assessment Document No 5. Limonene (1998)) indicates ambient levels of limonene in forest air of up to 12 µg/m^3, urban air up to 32 µg/m^3, and indoor air 480 µg/m^3.

  While it is clear that more research has been devoted to d-limonene than to α-terpinene, p-cymene, they are all are common terpenes in nature.

  Furthermore, none of these WHO and EFSA reports indicate any adverse effects from the current levels of exposure. The WHO (1998) report on additives notes that while there is exposure to these three terpenes there is not cause for concern and no ADI have been set.

- QRD 452 and QRD 460 are non-irritant or only mildly irritating to skin and eyes. The active substance QRD 460 and the plant protection product QRD 452 have been shown to be skin sensitizers in the LLNA. However QRD 452 was negative for skin sensitization under the conditions of the Buehler test. In contact with air d-limonene can break down to form small amounts of oxidation products which are known to be skin sensitizers. Gloves made from chemically resistant material should therefore be used when handling the plant protection product together with suitable protective clothing to avoid skin contact.

- For the mixing/loading and application of QRD 452, use of appropriate Personal Protective Equipment and hygiene measures is recommended. In the case of glasshouse applications it is recommended that appropriate respiratory equipment is also used.

Taking the results from all models as a whole and considering the nature and occurrence of the terpene components of QRD 460 it is concluded that uses of the product QRD 452 according to the proposed GAP and following label recommendations regarding PPE and hygiene measures will not result in any unacceptable risk to operators.

IIIA 7.3.3 MEASUREMENT OF OPERATOR EXPOSURE – (MIXER/LOADER/APPLICATOR)

Measurement of operator exposure is not required since model calculations predict the systemic exposure to be acceptable when appropriate PPE is worn.

IIIA 7.4 Bystander exposure

IIIA 7.4.1 ESTIMATION OF BYSTANDER EXPOSURE ASSUMING PERSONAL PROTECTIVE EQUIPMENT IS NOT USED

Bystanders are defined as persons who are not occupationally involved in the application or application related activities. Therefore, the exposure is considered to be incidental and as a result is less frequent, of shorter duration and at a lower level compared to the operator.

It is assumed that bystanders will not be present in glasshouses during or immediately following applications of QRD 452. Bystander exposure for the proposed outdoor applications is considered below.

The potential routes of exposure for bystanders are *via* dermal and inhalation exposure. All three terpenoid components of the active substance QRD 460 are extremely volatile by nature and QRD 460 is likely to degrade rapidly in air to form smaller, naturally occurring molecules. Please refer to point IIA 7.10 of the QRD 460 dossier for full details on the rate and route of degradation in air. For field applications it is considered that the rapid dissipation and dilution into the environment will mean that any inhalation exposure would be short-lived. Dermal exposure of bystanders may occur following drift. Such exposure is likely to be brief and unlikely to occur repeatedly to the same individual. However, both exposures were calculated and added to give a total systemic exposure.

Bystander exposure has been assessed according to EUROPOEM II[10]. Calculations of exposure and risk for the relevant outdoor use scenarios in table IIA 7.3-1 are presented. The maximum application rate of 1.523 kg a.s./ha and the minimum water volume of 400 L/ha have been used in the calculations. For details of the calculations refer to Appendix I.

Table IIIA 7.4.1-1: Estimated bystander exposure values and % of the MSDI

	Total systemic exposure (mg/day)	**% of MSDI (34.5 mg/day)**
Downwards application	1.67	5

The applied approach provides a conservative potentially 'worst-case' assessment of the exposure risk for incidental bystanders. The calculated exposure to QRD 452 demonstrates that there is no undue risk to incidental bystanders.

IIIA 7.4.2 MEASUREMENT OF BYSTANDER EXPOSURE

Measurement of bystander exposure is not required since model calculations predict the systemic exposure to be well within the acceptable exposure level.

IIIA 7.5 Worker exposure

IIIA 7.5.1 ESTIMATION OF WORKER EXPOSURE ASSUMING PERSONAL PROTECTIVE EQUIPMENT IS NOT USED

The type of crop, product type and the time point of application of QRD 452 mean re-entry activities such as crop inspection, pruning and harvesting may lead to some worker exposure. This applies to both glasshouse and field uses. Given the rapid breakdown of the product and the natural background levels of the terpenes to which workers are exposed in daily life, no harvest interval is proposed although as a standard rule treated areas should not be entered before the spray deposit on plant surfaces has dried.

The routes of exposure during post-application activities are analogous to the operator, i.e. dermal and inhalation but the sources are different e.g. contact with foliage.

<u>Dermal Exposure</u>

Treated areas should not be entered before the spray deposit on plant surfaces has dried. There are no dislodgeable foliar residues (DFR) data available for QRD 452. However residue studies conducted on tomato, mustard greens and primroses have demonstrated that multiple applications of QRD 452 or the original plant extract product result in no detection of residues even shortly after application (samples taken immediately following application) and no accumulation of residues over multiple applications. Therefore the dislodgeable foliar residue will be zero and hence there is no potential for dermal exposure. For full details please refer to point IIA 6.3 of the dossier on QRD 460. For this reason dermal worker exposure calculations are not presented and it can be concluded that re-entry worker exposure via the dermal route will be negligible.

[10] The development, maintenance and dissemination of generic European database and predictive exposure models to plant protection products. FAIR3 CT96-1406. Draft final report 2002

Inhalation Exposure

All three terpenoid components of the active substance QRD 460 are extremely volatile by nature and QRD 460 is likely to degrade rapidly in air to form smaller, naturally occurring molecules. Please refer to point IIA 7.10 of the QRD 460 dossier for full details on the rate and route of degradation in air.

For field applications it is considered that the rapid dissipation and dilution into the environment will mean that inhalation is not a significant exposure route for re-entry workers.

Using standard assumptions a maximum PEC_{AIR} in glasshouses of 0.043 mg/L (43 mg/m^3) has been calculated. However it is considered that this value is not appropriate for the estimation of worker exposure following glasshouse applications. All evidence from modelling, the literature and anecdotal evidence suggests that none of the terpenoid constituents of QRD 460 persist in the air and all are rapidly broken down. This means that the PEC_{AIR} value as calculated is worst case and any potential for inhalation exposure will reduce rapidly but this cannot be quantified precisely with the information available.

Since re-entry workers would not be present in glasshouses during spray application and would only enter the treated area some time later after spray deposits had dried it is reasonable to assume that air concentrations of QRD 460 would already have decreased significantly to form smaller molecules, naturally occurring in the air. This matches the anecdotal evidence from naturally occurring terpenoids such as d-limonene in oranges where the citrus fragrance dissipates rapidly after breaking the orange skin or slicing the fruit. It also matches anecdotal evidence from the use of d-limonene where it is used as a fragrance and the scent disappears after a few minutes.

There is no evidence that any of the constituents of QRD 460 persist in air. The models suggest that they all break down rapidly via hydroxyl radicals, ozone and nitrate radicals in a matter of minutes or a few hours and due to the nature of their chemistry as terpenoids, it is commonly accepted that they and their break down components will present no significant risk to the atmospheric environment. Anecdotal evidence from natural foodstuffs containing these terpenoids and from their use as fragrances in household items supports this position.

Hence, no adverse effects upon the health of workers who may be exposed to QRD 452 following re-entry into treated crops would be expected.

IIIA 7.5.2 ESTIMATION OF WORKER EXPOSURE ASSUMING PERSONAL PROTECTIVE EQUIPMENT IS USED

Addressed under point IIIA 7.5.1 above.

IIIA 7.5.3 ESTIMATION OF WORKER EXPOSURE ASSUMING PERSONAL PROTECTIVE EQUIPMENT IS USED AND USING DATA GENERATED ON DISLODGEABLE RESIDUES UNDER THE PROPOSED CONDITIONS OF USE

Addressed under point IIIA 7.5.1 above.

IIIA 7.5.4 MEASUREMENT OF WORKER EXPOSURE

Measurement of worker exposure is not required since it can be concluded that systemic exposure will be well within the acceptable levels.

IIIA 7.6 Dermal absorption

Dermal absorption has not been determined for QRD 452. Worst case values of 100% dermal absorption have therefore been used for the assessment.

IIIA 7.6.1 DERMAL ABSORPTION, *IN VIVO* IN THE RAT

See point IIIA 7.6

IIIA 7.6.2 COMPARATIVE DERMAL ABSORPTION, *IN VITRO* USING RAT AND HUMAN SKIN

See point IIIA 7.6

IIIA 7.7 Dislodgeable residues

IIIA 7.7.1 DISLODGEABLE RESIDUES – FOLIAR

This is not an EC data requirement.

IIIA 7.7.2 DISLODGEABLE RESIDUES – SOIL

This is not an EC data requirement.

IIIA 7.7.3 DISLODGEABLE RESIDUES INDOOR SURFACE RE-VOLATILIZATION

This is not an EC data requirement.

IIIA 7.8 Epidemiology

This is not an EC data requirement.

IIIA 7.9 Data on formulants

IIIA 7.9.1 MATERIAL SAFETY DATA SHEET FOR EACH FORMULANT

CONFIDENTIAL information - data provided separately (Document J)

IIIA 7.9.2 AVAILABLE TOXICOLOGICAL DATA FOR EACH FORMULANT

CONFIDENTIAL information - data provided separately (Document J)

IIIA 7.10 Domestic animal/livestock safety

This is not an EC data requirement.

IIIA 7.11 Other/special studies

This is not an EC data requirement.

REFERENCES

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
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IIIA 7.1.2	[redacted]	2008b	QRD 416: Acute dermal toxicity study in rats. [redacted]. Laboratory Report No. 11784-08, 9 July 2008. GLP, Unpublished.	Y	AQ
IIIA 7.1.3	[redacted]	2009	QRD 452: Acute inhalation toxicity study in rats. [redacted]. Laboratory Report No. 12566-08, 4 March 2009. GLP, Unpublished	Y	AQ
IIIA 7.1.4	[redacted]	2009a	QRD 452: Acute dermal irritation study in rabbits. [redacted]. Laboratory Report No. 12568-08, 4 February 2009. GLP, Unpublished.	Y	AQ
IIIA 7.1.5	[redacted]	2009b	QRD 452: Acute eye irritation study in rabbits. [redacted]. Laboratory Report No. 12567-08, 2 February 2009. GLP, Unpublished	Y	AQ
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IIIA 7.3 IIIA 7.3.2 Also submitted for IIA 5.1	WHO	2005	Evaluation of Certain Food Additives. WHO Technical Report Series No. 928. 63rd Report of the Joint FAO/WHO Expert Committee on Food Additives. Published	N	--
IIIA 7.3.2	Hakim et.al	2002	Development of a questionnaire and a database for assessing dietary d-limonene intake, Public Health Nutrition: 5(6A), 939–945. Not GLP. Published	N	--

APPENDIX I: DETAILED EXPOSURE CALCULATIONS

Table I-1: Dutch Glasshouse model – 1.523 kg a.s/ha application rate

OPERATOR EXPOSURE				DUTCH GREENHOUSE MODEL	
form	QRD 452 EC			Application including mixing and loading	
a.s.	QRD 460				
Parameter		**Value**	**Unit**	**References, comments**	
MANUAL SPRAYING in greenhouses					
AR	Application rate	1.532	kg a.s./ha	summary of intended uses	
A	Area treated	1	ha/ day	Dutch model	
Inhalation Exposure				without PPE	
SV	Surrogate Exposure Value	1	mg a.s./ kg a.s.	For dusting see note* (Dutch model)	
Inhalation Exposure (without PPE)		**1.532**	mg a.s./ day	IE = SV x AR x A	
Inhalation Exposure (with PPE)				with PPE	
	PPE-factor	10		default: 10	
Inhalation Exposure (with PPE)		**0.1532**	mg a.s./ day	IE(PPE) = (1/PPE factor) x IE	
Dermal Exposure				without PPE	
SV	Surrogate Exposure Value	200	mg a.s./ kg a.s.	For dusting see note* (Dutch model)	
Dermal Exposure		**306.4**	mg a.s./ day	DE = SV x AR x A	
Dermal Exposure (with PPE)				with PPE	
	PPE-factor	10		default (gloves & coverall): 10	
Dermal Exposure (with PPE)		**30.64**	mg a.s./ day	DE(PPE) = (1/PPE-factor) x DE	
Internal exposure					
IA	Inhalation Absorption	100	%		
DA	Dermal Absorption	100	%		
	AOEL	34.50	mg a.s./ day	based on 70 kg bw	
		Without PPE	**With PPE**		
	Internal exposure	[mg a.s. / day]	[mg a.s. / day]		
	Inhalation	1.5320	0.1532	IE(int) = IE x (IA/100)	
	Dermal	306.4000	30.6400	DE(int) = DE x (DA/100)	
	Total	**307.9320**	**30.7932**	**sum**	
	%AOEL				
	Inhalation	4	0	%AOEL = 100 x IE(int) / AOEL	
	Dermal	888	89	%AOEL = 100 x DE(int) / AOEL	
	Total	**893**	**89**	**sum**	

Table I-2: UK POEM Tractor Mounted Hydraulic – 1.523 kg a.s/ha application rate, no PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles				
Product	**QRD 452**		Active substance	**QRD 460**	
Formulation type	organic solvent-based		a.s. concentration	**152.3**	mg/ml
Dermal absorption from product	**100**	%	Dermal absorption from spray	**100**	%
Container	5 litres 45 or 63 mm closure				
PPE during mix/loading	None		PPE during application	None	
Dose	**10**	l/ha	Work rate/day	**50**	ha
Application volume	**400**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	5	litres
Hand contamination/operation	0.01	ml
Application dose	10	litres product/ha
Work rate	50	ha/day
Number of operations	100	/day
Hand contamination	1	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	1	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles					
Application volume	400	spray/ha				
Volume of surface contamination	10	ml/h				
Distribution	Hands		Trunk		Legs	
	65%		10%		25%	
Clothing	None		Permeable		Permeable	
Penetration	100%		5%		15%	
Dermal exposure	6.5		0.05		0.375	ml/h
Duration of exposure	6	h				
Total dermal exposure to spray	41.55	ml/day				

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	1	ml/day	41.55	ml/day
Concen. of a.s. product or spray	152.3	mg/ml	3.8075	mg/ml
Dermal exposure to a.s.	152.3	mg/day	158.201625	mg/day
Percent absorbed	100	%	100	%
Absorbed dose	152.3	mg/day	158.201625	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.01	m³/h
Duration of exposure	6	h
Concentration of a.s. in spray	3.8075	mg/ml
Inhalation exposure to a.s.	0.22845	mg/day
Percent absorbed	100	%
Absorbed dose	0.22845	mg/day

PREDICTED EXPOSURE

Total absorbed dose	310.730075	mg/day
Operator body weight	60	kg
Operator exposure	5.178834583	mg/kg bw/day

Table I-3: UK POEM Hand-held Application – 1.523 kg a.s/ha application rate, no PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Product	**QRD 452**	Active substance	**QRD 460**	
Formulation type	organic solvent-based	a.s. concentration	**152.3**	mg/ml
Dermal absorption from product	**100** %	Dermal absorption from spray	**100**	%
Container	5 litres 45 or 63 mm closure			
PPE during mix/loading	None	PPE during application	None	
Dose	**10** l/ha	Work rate/day	**1**	ha
Application volume	**400** l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	5	litres
Hand contamination/operation	0.01	ml
Application dose	10	litres product/ha
Work rate	1	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.27	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target					
Application volume	400	spray/ha				
Volume of surface contamination	50	ml/h				
Distribution	Hands		Trunk		Legs	
	25%		25%		50%	
Clothing	None		Permeable		Permeable	
Penetration	100%		20%		18%	
Dermal exposure	10		5		4.5	ml/h
Duration of exposure	6	h				
Total dermal exposure to spray	102	ml/day				

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.27	ml/day	102	ml/day
Concen. of a.s. product or spray	152.3	mg/ml	3.8075	mg/ml
Dermal exposure to a.s.	41.121	mg/day	388.365	mg/day
Percent absorbed	100	%	100	%
Absorbed dose	41.121	mg/day	388.365	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	m³/h
Duration of exposure	6	h
Concentration of a.s. in spray	3.8075	mg/m³
Inhalation exposure to a.s.	0.4569	mg/day
Percent absorbed	100	%
Absorbed dose	0.4569	mg/day

PREDICTED EXPOSURE

Total absorbed dose	429.9429	mg/day
Operator body weight	60	kg
Operator exposure	7.165715	mg/kg bw/day

Table I-4: UK POEM Tractor Mounted Hydraulic – 1.523 kg a.s/ha application rate, with PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Product	**QRD 452**	Active substance	**QRD 460**	
Formulation type	organic solvent-based	a.s. concentration	**152.3**	mg/ml
Dermal absorption from product	**100** %	Dermal absorption from spray	**100**	%
Container	5 litres 45 or 63 mm closure			
PPE during mix/loading	Gloves	PPE during application	Gloves	
Dose	**10** l/ha	Work rate/day	**50**	ha
Application volume	**400** l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	5	litres
Hand contamination/operation	0.01	ml
Application dose	10	litres product/ha
Work rate	50	ha/day
Number of operations	100	/day
Hand contamination	1	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.1	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles				
Application volume	400	spray/ha			
Volume of surface contamination	10	ml/h			
Distribution	Hands		Trunk	Legs	
	65%		10%	25%	
Clothing	Gloves		Permeable	Permeable	
Penetration	10%		5%	15%	
Dermal exposure	0.65		0.05	0.375	ml/h
Duration of exposure	6	h			
Total dermal exposure to spray	6.45	ml/day			

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.1	ml/day	6.45	ml/day
Concen. of a.s. product or spray	152.3	mg/ml	3.8075	mg/ml
Dermal exposure to a.s.	15.23	mg/day	24.558375	mg/day
Percent absorbed	100	%	100	%
Absorbed dose	15.23	mg/day	24.558375	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.01	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	3.8075	mg/ml
Inhalation exposure to a.s.	0.22845	mg/day
Percent absorbed	100	%
Absorbed dose	0.22845	mg/day

PREDICTED EXPOSURE

Total absorbed dose	40.016825	mg/day
Operator body weight	60	kg
Operator exposure	0.666947083	mg/kg bw/day

Table I-5: UK POEM Hand-held Application – 1.523 kg a.s/ha application rate, with PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target		
Product	**QRD 452**	Active substance	**QRD 460**
Formulation type	organic solvent-based	a.s. concentration	**152.3** mg/ml
Dermal absorption from product	**100** %	Dermal absorption from spray	**100** %
Container	5 litres 45 or 63 mm closure		
PPE during mix/loading	Gloves	PPE during application	Gloves and impermeable coveral
Dose	**10** l/ha	Work rate/day	**1** ha
Application volume	**400** l/ha	Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	5	litres
Hand contamination/operation	0.01	ml
Application dose	10	litres product/ha
Work rate	1	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.027	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	400	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	Gloves	Impermeable	Impermeable	
Penetration	10%	5%	5%	
Dermal exposure	1.25	0.625	1.25	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	18.75	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.027	ml/day	18.75	ml/day
Concen. of a.s. product or spray	152.3	mg/ml	3.8075	mg/ml
Dermal exposure to a.s.	4.1121	mg/day	71.390625	mg/day
Percent absorbed	100	%	100	%
Absorbed dose	4.1121	mg/day	71.390625	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	3.8075	mg/ml
Inhalation exposure to a.s.	0.4569	mg/day
Percent absorbed	100	%
Absorbed dose	0.4569	mg/day

PREDICTED EXPOSURE

Total absorbed dose	75.959625	mg/day
Operator body weight	60	kg
Operator exposure	1.26599375	mg/kg bw/day

Table I-6: German Model Tractor Field Crop – 1.523 kg a.s/ha application rate

= FIELD CROP TRACTOR MOUNTED =

	Treated area per day	**A =**	**20**	**ha/d**	at BBA = 20
	Use rate	**R =**	**1.523**	**kg a.i./ha**	

Mixing/loading of the product [mg/person per kg a.				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	**solid: WG**	I*a = 0,001	D*a/c = 0,06
I*m	0.0006	0.07	0.008	D*a/h = 0,38	D*a/b = 1,6
D*m/h	2.4	6	2		

Estimated inhalation exposure:

Im = I*m x R x A	**0.008**	1.523	20	0.24368 mg/pers. x d
Ia = I*a x R x A	0.001	1.523	20	0.03046 mg/pers. x d
		I, in total =		**0.27414 mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A	**2**	1.523	20	60.92 mg/pers. x d
Da/h = D*a/h x R x A	0.38	1.523	20	11.5748 mg/pers. x d
Da/c = D*a/c x R x A	0.06	1.523	20	1.8276 mg/pers. x d
Da/b = D*a/b x R x A	1.6	1.523	20	48.736 mg/pers. x d
		D, in total =		**123.0584 mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**		
Im =	0.24368	-	1		0.24368 mg/pers. x d
Ia =	0.03046	-	1		0.03046 mg/pers. x d
					0.27414 mg/pers. x d

Estimated derm. exp.

Dm/h =	60.92	SS 110	0.01		0.6092 mg/pers. x d
Da/h =	11.5748	SS 120	0.01		0.115748 mg/pers. x d
Da/c =	1.8276	SS 420	0.5		0.9138 mg/pers. x d
Da/b =	48.736	SS 220	0.05		2.4368 mg/pers. x d
					4.075548 mg/pers. x d

		Estimated exposure		**Systemic exposure**	
	abs. rate	**without PPE**	**with PPE**	**without PPE**	**with PPE**
Inhalation: m/l	100%	0.24368	0.24368	0.24368	0.24368
Inhalation: appl.	100%	0.03046	0.03046	0.03046	0.03046
Dermal: m/l	100%	60.92	0.6092	60.92	0.6092
Dermal: appl.	100%	62.1384	3.466348	62.1384	3.466348
			mg/pers./d:	123.33254	4.349688
kg bw:	70		mg/kg bw/d:	1.76189343	0.0621384
syst. AOEL:	0.49		**% of AOEL:**	**359.570087**	**12.6813061**

Table I-7: Europoem II – 1.523 kg a.s/ha application rate, downward application

BYSTANDER EXPOSURE			EUROPOEM II MODEL	
form	QRD 452 EC		**Outdoor application**	
as	QRD 460			
Parameter		**Value**	**Unit**	**References, comments**
SPRAYING Process outdoor				
AR	Application rate	1.523	kg a.s. / ha	summary of intended uses
SV	Spray volume	400	L / ha	summary of intended uses
Inhalation Exposure				without PPE
	Default value			
SE	Surrogate Exposure Value	0.03	mL / m3	downwards: 0.03; upwards: 0.06 (EUROPOEM II)
T	Time of exposure	1	h	most probable estimation
RR	Respiratory rate	1.25	m3 / h	default
Inhalation Exposure		**0.1428**	mg a.s. / day	IE = (ARx1000/SVxSExTxRR
Dermal Exposure				
	Default value			
SE	Surrogate Exposure Value	0.005		downwards: 0.005; upwards with leaves: 0.05; upward without leaves: 0.15 (EUROPOEM II)
SA	Surface area bystander	2	m2	EUROPOEM II
Dermal Exposure		**1.523**	mg a.s./ day	DE = SE xSA X (AR x 100)
Internal exposure				
IA	Inhalation Absorption	100	%	
DA	Dermal Absorption	100	%	
	AOEL	34.5	mg a.s./ day	based on 70 kg bw
		Without PPE		
	Internal exposure	[mg a.s./ day]		
	Inhalation	0.1428		IE(int) = IE x (IA/100)
	Dermal	1.523		DE(int) = DE x (DA/100)
	Total	**1.666**		**sum**
	% AOEL			
	Inhalation	0.4		%AOEL = 100 x IE(int) / AOEL
	Dermal	4.4		%AOEL = 100 x DE(int) / AOEL
	Total	**5**		**sum**