M-457054-01-3

Dossier According to Directive 91/414/EEC

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

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

DOCUMENT MII, Section 5

FATE AND BEHAVIOUR IN THE ENVIRONMENT

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7. FATE AND BEHAVIOUR IN THE ENVIRONMENT

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

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

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

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

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

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

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

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

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

Due to the 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 adds nothing of significance to the natural exposure, it is believed that safety is confirmed and so no additional data is considered necessary.

This means that the standard EU registration approach for assessment of environmental concentrations would be inappropriate and so two specialised studies have been performed and presented here to confirm the volatile character of the activity of the three active substance components: α-terpinene, ρ-cymene, d- limonene. The two studies presented here under Section 5 environmental fate and behaviour are the aerobic rate of degradation in soil study and a natural water degradation study.

Other than these studies, models have been used with the appropriate physical and chemical endpoints. Also a literature review has been performed, discussed and the conclusions summarised here.

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

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

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

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

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

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

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

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

It is the purpose of this Section to characterize the likely degradation pathways of QRD 460 as well as the degradation rates and extent of degradation in three environmental compartments, namely, soil, water and air. This characterization is based on the use of predictive modelling considering particularly the fugacity of the terpenes individually and research reports from the open literature. In addition to a literature-based and predictive characterization of the environmental fate of the active substance, recent experimental results characterizing degradation of the QRD 460 components in soil and natural water matrices are included.

Due to the use of predictive modelling that requires parameters from Section 1 Physical chemical properties, where appropriate, each terpene has been addressed individually.

Reference is closely made to [redacted](2011) and its respective appendices and references and to the FOCUS Air guidelines, Pesticides in Air – Considerations for Exposure Assessment SANCO /10553/2006 Rev 2 June 2008 and the US-EPA's EPI Suite™ model which is also discussed in the FOCUS guideline.

The physical-chemical properties of the three terpenes in QRD 460, α-terpinene, p-cymene, and d-limonene, indicate high vapor pressures and high Henry's Law Constants (see Section 1). This means that the dominant environmental sink for these compounds is likely to be the atmosphere. Monoterpenes, as a class, are released from vegetation in large amounts to the air (Fehsenfeld *et al.* 1992 and Guenther *et al.* 1995) which supports the assumption that volatilization is the most important environmental dissipation pathway for these compounds. Once in the air, research publications and predictive modeling indicate they are degraded rapidly based on interactions with hydroxyl radicals, ozone and nitrate radicals, the latter at night. To confirm this position, the fugacity of the three terpene components of QRD 460 is firstly considered.

Fugacity models are useful for understanding the fate and behavior of chemicals in the environment (SANCO /10553/2006 Rev 2 Pesticides in Air – Considerations for Exposure Assessment). Fugacity (a measure of escaping tendency of molecules) can be used to calculate multi-media equilibrium partitioning of organic chemicals such as the subject terpenes. Level I fugacity modeling describes the equilibrium partitioning of a chemical between environmental compartments. It gives a picture of the general affinity of a chemical for the various pure phases present in the environment. Level II and Level III fugacity modeling are more complex and more environmentally relevant as they take into account degradation processes as well as other (advective) losses from the various compartments. Level III fugacity modeling, in particular, is a non-equilibrium, steady-state model which is most useful as it takes into account inter-media transport rates (i.e., the extent to which a chemical moves from one medium to another) as well as the extent of degradation. The Levels I, II and III fugacity model was developed to assess the fate of a chemical within a large geographical area (100,000 km^2 region). In this report, the model is being used to provide a general picture of how the terpenes comprising QRD 460 distribute and degrade within certain environmental compartments.

α-Terpinene
Fugacity (Multi-Media) Model

Level I fugacity modelling of α-terpinene, based on MacKay's multi-media model (Level 1 Fugacity Model version 3.00 September 2004), indicates that 92.4 % will partition to air with 0.174 % going to water, 7.21 % to soil and 0.160 % to sediment. Level I of MacKay's multimedia fate model describes a situation where a fixed quantity of the chemical is introduced in a closed system, under steady-state and equilibrium conditions. The Level I calculation is performed in a six-compartment environment (air, soil, water, sediment, suspended sediment and fish) according to a fugacity approach described by MacKay *et al*. 1996. This model has been evaluated by the FOCUS Working Group on Pesticides in Air (SANCO /10553/2006 Rev 2 Pesticides in Air – Considerations for Exposure Assessment). Level I gives a picture of the general affinity of chemicals to the various pure phases in the environment but does not include degradation and other processes.

The fugacity model contained in EPI Suite™ version 4.0 2009 is a Level III multimedia fate model using environmental parameters identical to those used in MacKay *et al*. 1992 . The model is reduced to four main compartments, namely, air, water, soil and sediment. Mass transport between the compartments via volatilization, diffusion, deposition and runoff are modelled. Importantly, the model is a steady-state, non-equilibrium model. Steady state conditions mean that the change in concentration of the chemical in each compartment with respect to time eventually approaches zero. Loss of chemical occurs through reaction and advection. Reaction is the biotic or abiotic degradation of the chemical that is calculated using user-specified or model-calculated half lives of the chemical in each of the four main compartments. Advection is the process in air, water and sediment which involves removal of the chemical from a given compartment though losses other than degradation.

The distribution of the chemical and the environmental compartments depends on how the chemical is introduced in Level III. For simulating application of α-terpinene to a crop, the model was run assuming deposition from spraying plants was 90% to the air (representing a combination of what deposited on the crop foliage and what remained in the air following application), 1 % drift to an adjacent water body and the remainder (9%) reaching the soil and not the crop canopy. These are conservative estimates and represent a worst case. For α-terpinene, the fugacity model outputs are provided in Table 7-1. Input parameters were based on estimations within EPI Suite™ except for vapour

pressure and water solubility which were selected from the α-terpinene database. The Henry's Law constant was calculated from these data. The complete EPI Suite™ modeling run can be found in [redacted] 2011.

Table 7-1. Fugacity model outputs for α-terpinene.

Compartment	Mass Amount (%)	Half Life (hours)	Reaction (%)	Advection (%)
Air	0.0211	0.00311	97.6	0.00438
Water	9.06	360	0.362	0.188
Soil	90.6	720	1.81	0
Sediment	0.353	3240	0.00157	0.000147

It is important to note that the main environmental compartment receiving α-terpinene was air which also degraded α-terpinene much, much faster than the soil, sediment and water compartments.

It should also be noted that the environmental compartment distribution in Level III is based on reaching steady state conditions and not equilibrium in a closed system. Therefore, α-terpinene entering the air at application and during compartmental exchanges will quickly degrade (termed "reaction"). Thus, at steady state, very little α-terpinene will be in the air because degradation in air is so rapid.

Persistence in the total system or DT_{100} was predicted to be only 20.8 hours, extremely rapid for a pesticide, because much of the α-terpinene will partition to air and be degraded very quickly via interaction with hydroxyl and nitrate radicals and with ozone (discussed further under Section 7.10 Fate in Air).

Note also that reaction processes were greater than advection processes in all compartments but particularly in air where the percentages were 97.6 and 0.00438 for reaction and advection, respectively. Overall, reaction and advection contributed 99.8 and 0.193 %, respectively. Because advection in air is a very minor process, α-terpinene will likely degrade in air on site rather than move off site.

p-Cymene
Fugacity (Multi-Media) Model

Following the same methodology as for α-terpinene, Level I MacKay modelling (Level 1 Fugacity Model version 3.00 September 2004) indicates that 88.4 % of p-cymene will partition to air with 0.321 % going to water, 11.1 % to soil and 0.246 % to sediment. Level I of MacKay's multimedia fate model describes a situation where a fixed quantity of the chemical is introduced in a closed system, under steady-state and equilibrium conditions. The Level I calculation is performed in a six-compartment environment (air, soil, water, sediment, suspended sediment and fish) according to a fugacity approach described by MacKay *et al*. 1996. This model has been evaluated by the FOCUS Working Group on Pesticides in Air (SANCO /10553/2006 Rev 2 Pesticides in Air – Considerations for Exposure Assessment). Level I gives a picture of the general affinity of chemicals to the various pure phases in the environment but does not include degradation and other processes.

The fugacity model in EPI Suite™ version 4.0 2009 is a Level III multimedia fate model using environmental parameters identical to those used in MacKay *et al*. 1992. The model is reduced to four main compartments, namely, air, water, soil and sediment. Mass transport between the compartments via volatilization, diffusion, deposition and runoff are modelled. Importantly, the model is a steady-state, non-equilibrium model. Steady state conditions mean that the change in concentration of the chemical in each compartment with respect to time eventually approaches zero. Loss of chemical occurs through reaction and advection. Reaction is the biotic or abiotic degradation of the chemical that is calculated using user-specified or model-calculated half lives of the chemical in each of the four main compartments. Advection is the process in air, water and sediment which involves removal of the chemical from a given compartment though losses other than degradation.

The distribution of the chemical and the environmental compartments depends on how the chemical is introduced in Level III. For simulating application of p-cymene to a crop, the model was run assuming deposition from spraying plants was 90% to the air (representing a combination of what deposited on the crop foliage and what remained in the air following application), 1 % drift to an adjacent water body and the remainder (9%) reaching the soil and not the crop canopy. For p-cymene, the fugacity model outputs are provided in the Table 7-2. Input parameters were based on estimations within EPI Suite™ except for vapour pressure and water solubility which were taken from the p-cymene database. The Henry's Law constant was calculated from these data. The complete EPI Suite™ modelling run can be found in [redacted] 2011.

Table 7-2. Fugacity model outputs for p-cymene.

Compartment	Mass Amount (%)	Half Life (hours)	Reaction (%)	Advection (%)
Air	41.2	17	77.9	19.1
Water	4.14	360	0.37	0.192
Soil	54.5	720	2.44	0
Sediment	0.161	3240	0.0016	0.00015

It is important to note that the main environmental compartment receiving p-cymene was air (see Level I) which also degraded p-cymene much faster than the soil, sediment and water compartments although not as fast as d-limonene and α-terpinene.

It is notable that the environmental compartment distribution in Level III is based on reaching steady state conditions and not equilibrium in a closed system.

Persistence in the total system or DT_{50} was predicted to be 46.4 hours, extremely rapid for a pesticide, because most of the p-cymene will partition to air and be degraded via interaction with hydroxyl radicals (discussed further under Section 7.10 Fate in Air) rapidly.

Note also that reaction processes were greater than advection processes in all compartments. Overall, reaction and advection contribute 80.7 and 19.3 %, respectively. It is also interesting to note that the steady-state concentration in air for p-cymene is higher than that predicted for d-limonene and α-terpinene. That is because the rate of degradation for p-cymene in air (17-hour half life as predicted by EPI Suite™ and used for the fugacity model calculations) is longer than the other two monoterpenes, but still extremely short compared to standard pesticides.

d-Limonene
Fugacity (Multi-Media) Model

As for α-terpinene and p-cymene, Level I MacKay modelling of d-limonene (Level 1 Fugacity Model version 3.00 September 2004) indicates that 84.9 % of d-limonene will partition to air, 0.319% to water, 14.5% to soil and 0.322 % to sediment. Level I of MacKay's model specifically describes a situation where a fixed quantity of the chemical is introduced in a closed system under equilibrium conditions. The Level I calculation is performed in a six-compartment environment (air, soil, water, sediment, suspended sediment and fish) according to a fugacity approach described by MacKay *et al.* 1996. Level I gives a picture of the general affinity of chemicals to the various pure phases in the environment but does not include degradation and other processes.

The fugacity model contained in EPI Suite™ version 4.0 2009 is a Level III multimedia fate model using environmental parameters identical to those used in MacKay *et al*. 1992. Note that this model has also been evaluated by the FOCUS Working Group on Pesticides in Air (SANCO /10553/2006 Rev 2 Pesticides in Air – Considerations for Exposure Assessment). The model is reduced to four main compartments, namely, air, water, soil and sediment. Mass transport between the compartments via volatilization, diffusion, deposition and runoff are

modeled. Importantly, the model is a steady-state, non-equilibrium model. Steady state conditions mean that the change in concentration of the chemical in each compartment with respect to time eventually approaches zero. Loss of chemical occurs through reaction and advection. Reaction is the biotic or abiotic degradation of the chemical that is calculated using user-specified or model-calculated half lives of the chemical in each of the four main compartments. Advection is the process in air, water and sediment which involves removal of the chemical from a given compartment though losses other than degradation.

The distribution of the chemical in the environmental compartments depends on how the chemical is introduced in Level III. For simulating application of d-limonene to a crop, the model was run assuming that spraying plants resulted in 90% to the air (representing a combination of what deposited on the crop foliage and what remained in the air following application), 1 % spray drift to an adjacent water body and the remainder (9%) reaching the soil and not the crop canopy. These are conservative estimates and represent a worst case. For d-limonene, with these assumptions, the fugacity model outputs are provided in the Table 7-3. Input parameters were based on estimations within EPI Suite™ except for vapour pressure and water solubility which were pre-selected from the d-limonene database and the Henry's Law constant which was calculated from vapour pressure and water solubility. The complete EPI Suite™ modelling run can be found in [redacted] 2011.

Table 7-3. Fugacity model outputs for d-limonene.

Compartment	Mass Amount (%)	Half Life (hours)	Reaction (%)	Advection (%)
Air	1.38	0.334	96	0.463
Water	5.69	360	0.368	0.191
Soil	92.7	720	3	0
Sediment	0.222	3240	0.00159	0.000149

It is important to note that the main environmental compartment receiving d-limonene was air (Level I modeling) which is also predicted to degrade d-limonene much, much faster than the soil, sediment and water compartments (Level III). It is also notable that the environmental compartment distribution in Level III is based on reaching steady state conditions and <u>not</u> equilibrium in a closed system.

Therefore, d-limonene entering the air at application and during compartmental exchanges will quickly degrade. Persistence in the total system or DT_{100} was predicted to be 33.6 hours, extremely short compared to most pesticides, because most of the d-limonene will partition to air and be degraded very quickly via interaction with hydroxyl and nitrate radicals and with ozone (discussed further under Section 7.10 Fate in Air).

Note also that reaction processes were greater than advective processes in all compartments but particularly in air where the percentages were 96.0 and 0.463 for reaction and advection, respectively. Overall, reaction and advection contribute 99.3 and 0.654 %, respectively. Because advection in air is a very minor process, d-limonene will largely degrade in air at the site of application rather than move off site.

IIA 7.1 Route of degradation in soil – laboratory studies

Introduction

From the fugacity models included in the introduction to this section, it is clear that QRD 460 exhibits the main environmental characteristic of rapidly partitioning into the air compartment by volatilisation, all three terpene components being extremely volatile in nature.

On this basis, the route and rate of degradation in soil have limited applicability to the environmental fate of QRD 460 when applied as a pesticide.

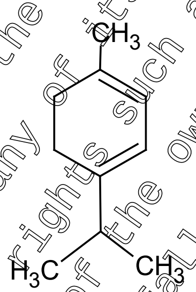
To ensure a rigorous assessment, a literature review has been conducted and further modelling considered in the following information summarised below, addressing each of QRD 460's constituents individually:

Fate of α-Terpinene in Soil

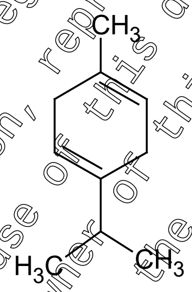
α-Terpinene is predicted to biodegrade rapidly under aerobic conditions, on a timescale of days to weeks, in four of the six BIOWIN 4.10 models contained within EPI Suite™ version 4.0. Ultimate biodegradation, i.e., conversion of α-terpinene to carbon dioxide (BIOWIN 3), is predicted to occur within weeks while initial steps of biodegradation (BIOWIN 4) are predicted to occur within days to weeks. In two of the models, BIOWIN 5 and 6, representing MITI (Japanese Ministry of International Trade and Industry) testing, α-terpinene was not considered to be readily biodegradable based on microbial oxygen uptake in the OECD 301C test. α-terpinene is not predicted to biodegrade quickly under anaerobic conditions (BIOWIN 7).

Although these predictive models provide an idea as to the degradability of α-terpinene, an actual aerobic soil degradation study has shown that the predominant dissipation pathway is volatility (see Section 7.2.1 Aerobic degradation) confirming the fugacity modeling. That is, α-terpinene was completely removed from soil in less than 48 hours. Thus, although the models predict rapid microbial degradation in soils and sewage sludge, it appears that α-terpinene is even more quickly removed via volatilization and subsequently degraded rapidly in the air (see Section 7.10 Fate in Air).

No reports of the biodegradation of α-terpinene were found in the open literature. However, the structural isomer, γ-terpinene, was studied (Misra *et al*. 1997). Chemical structures of the α and γ isomers of terpinene are depicted below showing how similar they are to each other.



α-terpinene



γ-terpinene

In soil slurries which were not autoclaved and azide-treated, complete removal of γ-terpinene occurred after 120 hours. In sterilized soils, about 74% of the starting γ-terpinene was recovered after 120 hours. The difference in recovered monoterpene between the microbially active samples and the controls was considered to be due to biodegradation. Because both d-limonene and γ-terpinene were readily degraded by indigenous soil microorganisms, α-terpinene should be readily biodegraded as well.

Fate of p-Cymene in Soil

p-Cymene is predicted to biodegrade rapidly under aerobic conditions, on a timescale of days to weeks, in four of the six BIOWIN 4.10 models contained within EPI Suite™ version 4.0. Ultimate biodegradation, i.e., conversion of p-cymene to carbon dioxide (BIOWIN 3), is predicted to occur within weeks while initial steps of biodegradation (BIOWIN 4) are predicted to occur within days to weeks. In two of the models, BIOWIN 5 and 6, representing MITI (Japanese Ministry of International Trade and Industry) testing, p-cymene was not considered to be readily biodegradable based on microbial oxygen uptake in the OECD 301C test. P-cymene is not predicted to biodegrade quickly under anaerobic conditions (BIOWIN 7).

Although these predictive models provide an idea as to the degradability of p-cymene, an actual aerobic soil degradation study has shown that the predominant dissipation pathway is volatility (see Section 7.2.1 Aerobic degradation) confirming the fugacity modeling. That is, p-cymene was completely removed from soil within 48 hours. Thus, although the models predict rapid microbial degradation in soils and sewage sludge, it appears that p-

cymene is even more quickly removed via volatilization and subsequently degraded rapidly in the air (see Section 7.10 Fate in Air).

The biodegradation potential of p-cymene was evaluated using the MITI test method (Ministry of international Trade and Industry, Japan; OECD 301C [test for ready biodegradability]) and reported by Klopman and Tu, 1997. Specifically, 100 mg/L of the test chemical is incubated with 30 mg/L of sludge for up to 28 days. Reported activity is described as final day biochemical oxygen demand (BOD), i.e., oxygen uptake. In the case of p-cymene, final day BOD was 88% indicating extensive biodegradation.

Bacteria that degrade p-cymene are relatively common (Eaton, 1997). They initiate catabolism of p-cymene by oxidizing the benzylic methyl group to form p-cumate (p-isopropylbenzoate). *Pseudomonas putida* F1 utilizes p-cymene by an 11-step pathway through p-cumate to isobutyrate, pyruvate and acetyl coenzyme A (Tehsenfeld *et al.* 1992). The microbial degradation pathway for p-cymene utilized by *P. putida* F1 is provided in Figures 7.1-1 and 7.1-2.

Figure 7.1-1 Initial pathway for the degradation of p-cymene in *Pseudomonas putida* F1 (Eaton, 1997).

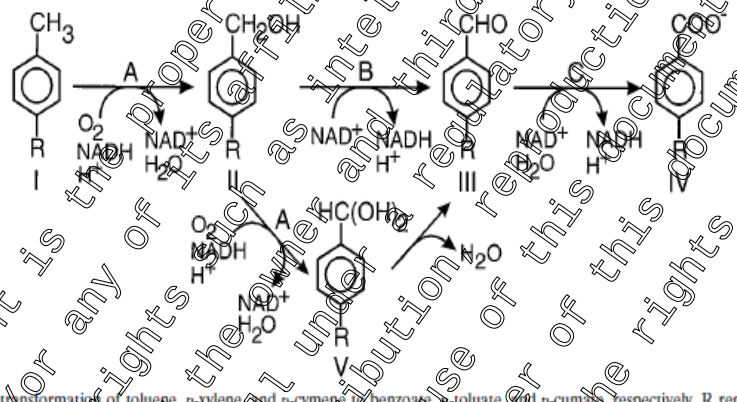


FIG. 1. Pathway for the transformation of toluene, *p*-xylene, and *p*-cymene to benzoate, *p*-toluate, and *p*-cumate, respectively. R represents the following: H, for toluene and metabolites; CH_3, for *p*-xylene and metabolites; and $CH(CH_3)_2$, for *p*-cymene and metabolites. Compounds are represented as follows: I, toluene, *p*-xylene, or *p*-cymene; II, benzyl alcohol, *p*-tolyl alcohol, or *p*-cumic alcohol; III, benzaldehyde, *p*-tolualdehyde, or *p*-cumic aldehyde; IV, benzoate, *p*-toluate, or *p*-cumate; and V, α,α′-dihydroxytoluene, α,α′-dihydroxy-*p*-xylene, or α,α′-dihydroxy-*p*-cymene. Enzymes are represented as follows: A, monooxygenase; B, alcohol dehydrogenase; and C, aldehyde dehydrogenase.

Figure 7.1-2 Initial pathway for the degradation of p-cymene in *Pseudomonas. putida* F1 (Eaton 1997).

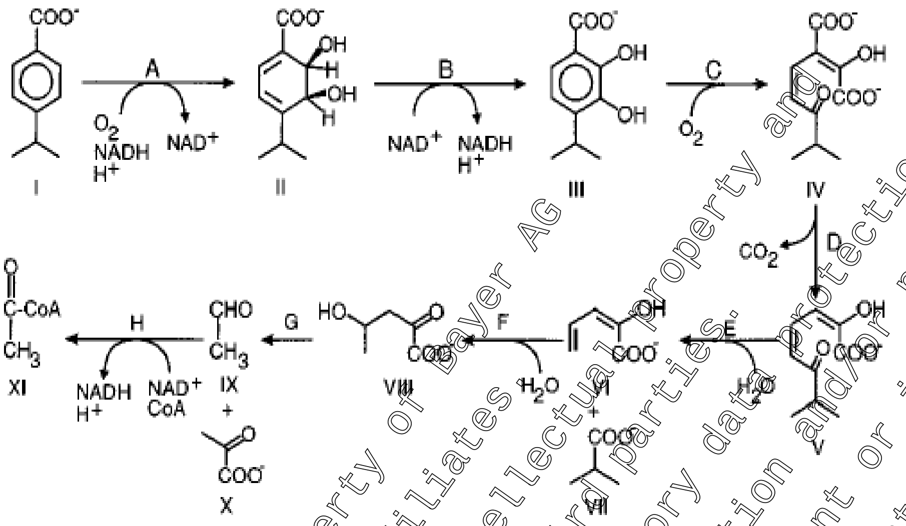


FIG. 1. Pathway for the catabolism of *p*-cumate. Chemicals are: I, *p*-cumate; II, *cis*-2,3-dihydroxy-2,3-dihydro-*p*-cumate; III, 2,3-dihydroxy-*p*-cumate; IV, 2-hydroxy-3-carboxy-6-oxo-7-methylocta-2,4-dienoate; V, 2-hydroxy-6-oxo-7-methylocta-2,4-dienoate; VI, 2-hydroxypenta-2,4-dienoate; VII, isobutyrate; VIII, 2-oxo-4-hydroxyvalerate; IX, acetaldehyde; X, pyruvate; and XI, acetyl-coenzyme A (CoA). Enzymes are: A, *p*-cumate 2,3-dioxygenase; B, 2,3-dihydroxy-2,3-dihydro-*p*-cumate dehydrogenase; C, 2,3-dihydroxy-*p*-cumate dioxygenase; D, 2-hydroxy-3-carboxy-6-oxo-7-methylocta-2,4-dienoate decarboxylase; E, 2-hydroxy-6-oxo-7-methylocta-2,4-dienoate hydrolase; F, 2-hydroxypenta-2,4-dienoate hydratase; G, 2-oxo-4-hydroxyvalerate aldolase; H, acetaldehyde dehydrogenase (acylating) (15–17, 72, 83, 87).

Fate of d-Limonene in Soil

As a starting point, the BIOWIN module within the EPA model EPI Suite™ was used to predict the degradability (both initial steps and complete degradation) of d-limonene. Specifically, the BIOWIN models were used to predict aerobic and anaerobic biodegradation of organic compounds in the presence of mixed populations of environmental microorganisms. There are seven different models within the BIOWIN suite. Biodegradation estimates are based upon fragment constants that were developed using both linear and non-linear regressions. The models were validated using an independent validation set of compounds. A more complete description of all seven models can be found in the On-Line BIOWIN User's Guide within the Help menu of EPI Suite™. The complete EPI Suite™ modelling run can be found in [redacted] 2011.

d-Limonene is predicted to biodegrade rapidly under aerobic conditions, on a timescale of days to weeks, in four of the six BIOWIN 4.10 models contained within EPI Suite™ 4.0. Ultimate biodegradation, i.e., conversion of d-limonene to carbon dioxide (BIOWIN 3), is predicted to occur within weeks while initial steps of biodegradation (BIOWIN 4) are predicted to occur within days to weeks. In two of the models, BIOWIN 5 and 6, representing MITI (Japanese Ministry of International Trade and Industry) testing, d-limonene was not considered to be readily biodegradable based on microbial oxygen uptake in the OECD 301C test. D-limonene is not predicted to biodegrade quickly under anaerobic conditions (BIOWIN 7).

Although these predictive models provide an idea as to the degradability of d-limonene, an actual aerobic soil degradation study has shown that the predominant dissipation pathway is volatility (see Section 7.2.1 Aerobic degradation) confirming the fugacity modeling. That is, d-limonene was completely removed from soil in less than 48 hours. Thus, although the models predict rapid microbial degradation in soils and sewage sludge, it appears that

d-limonene is even more quickly removed via volatilization and subsequently degraded rapidly in the air (see Section 7.10 Fate in Air).

Microbial degradation may be considered further, as follows.

There have been many reports about the biotransformation of limonene in pure microbial cultures. Five proposed pathways are illustrated in Figure 7.1-3.

Figure 7.1-3. Various pathways for degradation of d-limonene in microbial species (Van der Werf *et al.* 1999).

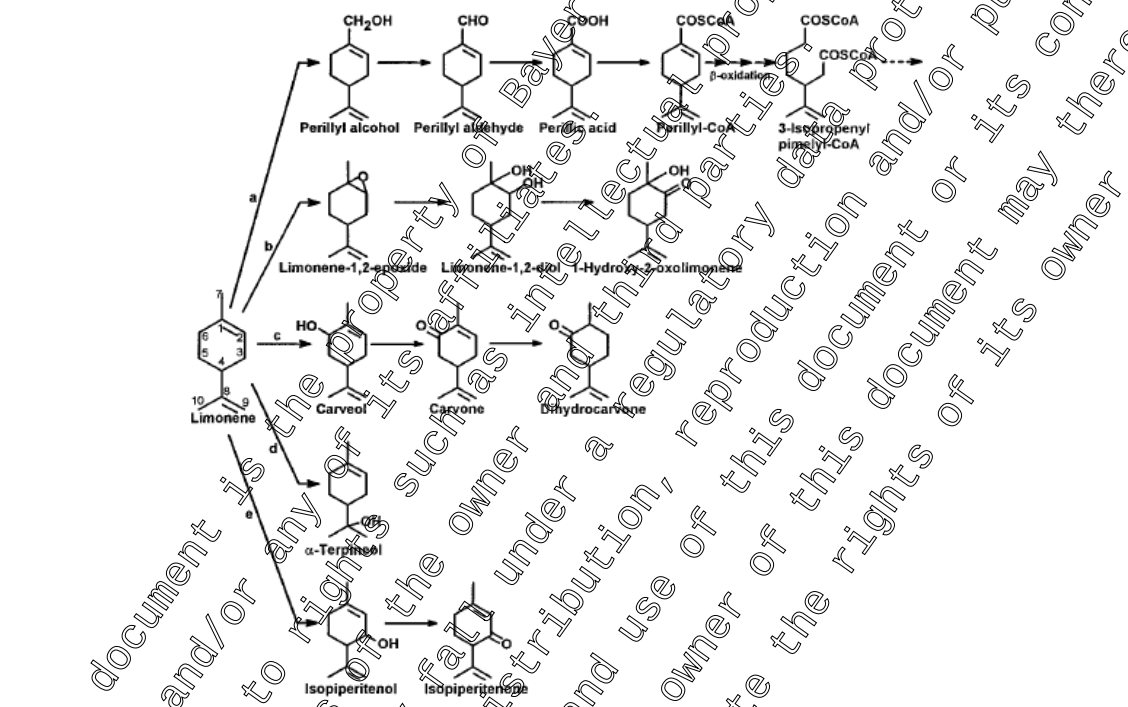
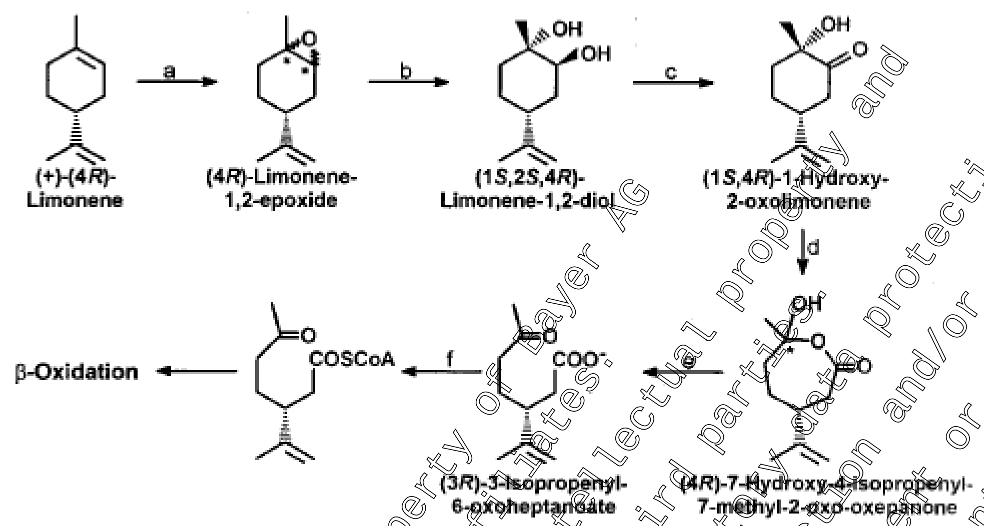


FIG. 3. Microbial biotransformation pathways for limonene. Route a is from references 8, 11, 12, 16, 18, 35, 38, and 52; route b is from references 1, 8, 16, 20, 34, 35, and 52; route c is from references 8, 16, 29, and 35; route d is from references 11, 12, 30, 33, 43, and 43; and route e is from references 29, 35, 51, and 52. The numbers in the limonene molecule refer to the carbon atom numbering of limonene.

Pathway (a) in Figure 7.1-3 is proposed for a strain of *Pseudomonas putida* PL where biotransformation is initiated by hydroxylation of limonene at the C-7 methyl group by a membrane-bound oxygenase resulting in the formation of perillyl alcohol. This initial transformation product is subsequently converted to perillyl aldehyde and perillic acid. Perillic acid is then oxidized in a fashion analogous to a fatty acid-β-oxidation reaction sequence resulting in the formation of 3-isopropenylpimelyl-CoA. Van der Werf *et al*. 1999 noted that their research group had isolated 56 bacteria that were able to grow on limonene as a sole source of carbon and energy which suggests that limonene is mineralized (i.e., completely metabolized to carbon dioxide). Interestingly, these authors had isolated a bacterial strain (*Rhodococcus erythropolis* DCL14) that metabolized (and mineralized) d-limonene via a different pathway (Figure 7.1-4).

Figure 7.1-4. Pathway for catabolism of d-limonene in *Rodococcus erythropolis* DCL14 (Van der Werf *et al*. 1999).



As illustrated, the degradation pathway for d-limonene in *R. erythropolis* DCL14 starts with attack of the cyclic double bond via an FAD- and NADH-dependent monooxygenase. A 1,2-epoxide hydrolase catalyzes the hydrolysis of the epoxide, forming a cis-dihydrodiol. The diol is then oxidized to a keto-alcohol by a dehydrogenase and is the substrate for a lactone-forming monooxygenase. The lactone formed is unstable and spontaneously rearranges to form an oxo acid which then undergoes β-oxidation that ultimately leads to mineralization or completed utilization (degradation) of d-limonene.

[redacted] (2011) refers to research on the biodegradation kinetics of limonene in soil-slurry and liquid culture systems. The 20% soil-slurry was prepared by mixing 2 grams of soil with 10 mL of distilled, deionized water in serum tubes. The tubes were flushed with pure oxygen and sealed. Limonene was directly injected into the tubes using a microsyringe. Incubations took place in the dark at 23 °C with continuous rotation of the tubes. CO_2 was determined by gas chromatography and limonene quantification was accomplished by liquid/liquid extraction with isooctane and gas chromatography using a flame ionization detector. Microbes used for liquid cultures were taken from enrichment cultures that had been semi-continuously fed with limonene and α-terpinene. Enriched cultures were added to serum tubes, flushed with oxygen and sealed. At predetermined times, duplicate tubes were harvested and analyzed for headspace CO_2, residual limonene and biomass concentrations. Biomass was measured gravimetrically or was estimated using either absorbance measurements at 500 nm or ATP measurements using a luminometer.

There was some reduction in limonene soil concentration in autoclaved, azide-treated soil (approximately20% after 120 hours), thought to be due to adsorption but, in contrast, limonene was completely removed from microbially active soils after ~80 – 120 hours of incubation. In liquid cultures, limonene was completely removed after approximately 50 – 70 hours of incubation. There was a concomitant increase in microbial biomass and CO_2 production was the mirror image of the limonene concentration profile. In summary then, limonene was readily and rapidly degraded by indigenous soil microorganisms from both soil and liquid mixed-microbial cultures.

[redacted] (2011) also notes that biodegradation has also been assessed under anaerobic conditions; however, there was no indication of any degradation of limonene.

When associated with the soil compartment, d-limonene is expected to have low to very low mobility based on its physical/chemical properties because its Koc is predicted to be 6324 L/kg (EPI Suite™ modelling run can be found in [redacted] 2011). Furthermore, its Henry's Law Constant (1.28 x 10^{-2} atm-m^3/mole) indicates that d-limonene will rapidly volatilize from both dry and moist soils. The high propensity of any remaining d-limonene to adsorb to soil may retard the volatilization process.

Conclusion

From the literature review and the fugacity modelling, it is clear that the fate of QRD 460 has limited relevance in the soil compartment.

At the request of the Rapporteur and to further support the position that the fate of QRD 460 in soil is of low relevance, one aerobic rate of degradation soil study was performed on the individual terpenes in QRD 460, see Section 7.2.1. This is a non-standard study because the terpenes in QRD 460 volatilise rapidly and this places constraints on the methodology of all the usual guideline studies.

The soil degradation study was performed to GLP and concluded that all QRD 460 components, α-terpinene, p-cymene and d-limonene, evaporated rapidly from the soil into the trapping solution. The DT_{50} of all three test items, α-terpinene, p-cymene and d-limonene, was calculated as <24 hours. The DT_{90} which was also the DT_{100} was <48 hours.

This study clearly demonstrates that the fate of QRD 460 in soil is of low relevance to its environmental fate after pesticidal use, and that further consideration of QRD 460 fate in soil should not be necessary.

IIA 7.1.1 Anaerobic degradation

Not relevant for Terpenoid blend (α-terpinene, p-cymene, d-limonene) QRD 460, see 7.1. above.

IIA 7.1.2 Soil photolysis

Not relevant for Terpenoid blend (α-terpinene, p-cymene, d-limonene) QRD 460, see 7.1 above.

IIA 7.2 Rate of degradation in soil(s) – laboratory studies

IIA 7.2.1 Aerobic degradation of the active substance in soils at 20°C

Report:	IIA 7.2.1/01 [redacted] F 2010, d-Limonene, p-Cymene, α-Terpinene: Aerobic Rate of Degradation of the Active Components of QRD 460 in Soil, [redacted] Study # 1145.902.760, 20 December 2010

Guidelines

OECD guideline # 307 (2002)

GLP: Yes

Executive Summary

The aerobic soil degradation of α-terpinene, p-cymene and d-limonene was studied in one representative soil. Test vessels containing 100 g (dry weight) were pre-incubated under aerobic conditions for four days prior to application. The three test substances were applied individually to achieve final nominal concentrations of approximately [redacted] mg/kg α-terpinene, [redacted] mg/kg p-cymene and [redacted] mg/kg d-limonene, this reflects the relative proportion of each terpene in the active substance QRD 460. A continuous flow-through test system was used at a temperature of 20 ± 2°C in the dark. Aerobic conditions were maintained by continuously bubbling moistened air through the water layer. Each replicate was equipped with a trap containing iso-octane as trapping solution to collect volatile test item or possible degradation products. Samples were analyzed after 0 and 7 hours, and 1, 2 and 3 days after application. The trap of the respective sample was analyzed too.

Duplicate samples for each test item were analyzed at each sampling interval. The soil was extracted with acetonitrile. The acetonitrile fraction was further extracted by liquid/liquid extraction with hexane. The hexane was concentrated and then analyzed by GC. The trapping solution was analyzed by GC without any further treatment.

All test items evaporated rapidly from the soil into the trapping solution. The DT_{50} of all three test items was calculated as <24 hours. The DT_{90} which was actually also the DT_{100} was <48 hours.

Materials

Test material	α-terpinene	p-cymene	d-limonene
Description:	Not reported	Not reported	Not reported
CAS number	99-86-5	99-87-6	5989-27-5
Lot/Batch #:	812097	812108	810763
Purity:	92.6%	99.1%	95.0%
Stability of test compound:	Not determined	Not determined	Not determined

Application vehicle:	Acetonitrile
Soil	One European soil (Sevelen, Switzerland)
Name:	Sevelen
Sampling location:	Switzerland
Date of collection:	21 September 2010
Sampling depth:	10 - 20 cm
Storage conditions:	Refrigerated until 5 days before use then acclimatised to test temperature
Particle size (% w/w):	
Sand (2000-50 µm)	54.3
Silt (50-2 µm)	37.9
Clay (< 2 µm)	7.8
Texture (USDA)	Sandy Loam
pH (water)	7.1
Organic matter (% w/w)	1.57
CEC (meq/100 g soil)	9.35
Moisture at pF 2 (% w/w)	29.78
Biomass (mg carbon/100 g soil):	
Pre-study	21.0
Start of study	22.5
Day 7	, α-terpinene 22.5, p-cymene 22.5, d-limonene 22.5

Study Design and Methods

Experimental Design

Parameter	**Description**
Duration of the test	4 days (26 – 29 October 2010). Last analytical measurement 15 November 2010
Soil condition	Fresh soil, passed through a 2 mm sieve prior to use
Soil sample weight	100 g (dry weight) per replicate

Parameter		Description
Test concentration (mg ai/kg soil (dry weight))		α-terpinene [redacted] p-cymene d-limonene
Control conditions		Not applicable
Number of replicates		2
Test apparatus		500 mL glass screw cap vessels. Humidified air flow-through system
Traps for CO_2 & organic volatiles		One 2,2,4-trimethylpentane (iso-octane) trap
Test material application:	Identity of solvent	Acetonitrile
	Volume of test solution used/replicate (µL)	α-terpinene [redacted] p-cymene d-limonene
	Application method	Direct application followed by shaking to mix
	Evaporation of application solvent	Not reported
Indication of test material adsorbing to walls of test apparatus		Not reported
Experimental conditions:	Temperature	20 ± 2°C
	Moisture content	50% maximum water holding capacity
	Moisture maintenance method	Not required
	Continuous darkness (Yes/No)	Yes
Sampling intervals:	Aerobic	Duplicate samples 0 and 7 hours 1, 2 and 3 days
	Untreated soils for biomass	Pre-study, at start of study and on day 7
Soil sampling procedures		Entire soil sample removed and extracted with acetonitrile followed by hexane
Collection of CO_2 and volatile organics		Total volume of the iso-octane trapping solution was determined. Sub samples were directly analysed using GC-FID without any further concentration or clean up
Sample storage before analysis		Details not reported

Analytical Procedures

The entire soil sample was extracted by adding 80 mL of acetonitrile (ACN) to the soil. The samples were taken by hand and were thereafter centrifuged. The supernatant was decanted in an intermediate flask. The extraction was repeated two more times. The supernatants of each extraction were combined and transferred to a separating funnel. Amounts of 50 mL of deionized water and 50 mL of hexane were also added to the separating funnel. After vigorously shaking the funnel the lower phase consisting of water-ACN was drained off. The hexane phase was collected in a 250 mL round-glass flask. The water-ACN phase was extracted again with 50 mL of hexane. After shaking and separating the phases the hexane phase was collected as described above. The combined hexane phases were concentrated by rotary to a final volume of approximately 10-20 mL. The definitive volume was recorded. A sub-sample of the concentrated soil extract was analyzed for test item and degradation products using GC-FID.

The total volume of the iso-octane trapping solution was determined for each replicate. Sub-samples were directly analyzed by GC-FID, without any further concentration or clean-up.

Results and Discussion

Mass Balance

The study was performed with non-radio labelled test material. Therefore, no mass balance can be given.

Extractable Residues

The soil extract of hour 0, i.e. directly after application showed less α-terpinene than originally applied. In total, 0.79 and 1.20 mg a.i./kg were found in the soil extracts. A minor amount of p-cymene was detected as degradation product. The level of p-cymene did not exceed 0.06 mg a.i./kg in both replicates of hour 0. Seven hours after application, the level of α-terpinene in soil extract was already below the LOQ of 0.4 mg a.i./kg. The level on day 1 was again <LOQ. From day 2 onwards, no residue was detectable.

In the soil extract of p-cymene of hour 0, 0.55 and 0.56 mg a.i./kg of the applied 0.59 mg a.i./kg were found. No degradation products were detected. 7 hours after application, the soil extract of one replicate contained 0.10 mg a.i./kg p-cymene, whereas the other replicated showed a concentration <LOQ (<0.04 mg a.i./kg). On day 1, both of the replicates with p-cymene showed concentrations <LOQ and from day 2 onwards there was no detectable residue.

In the soil extract of d-limonene of hour 0, 0.51 and 0.53 mg a.i./kg were found. These calculated values were slightly higher than the applied 0.46 mg a.i./kg of d-limonene. No degradation products were detected. By 7 hours after application, both replicates showed a concentration <LOQ (0.04 mg a.i./kg). From day 2 onwards there was no detectable residue.

Volatile Test Item and/or Degradation Products

For all three test items levels of volatile test item and/or degradation products increased from 7 hours to one day after application. Thereafter amounts decreased. The test item and their degradation products disappeared from the soil into the trapping solution. Due to the continuous aeration, the test items were pushed out of the trapping solution with ongoing time.

Degradation Kinetics

The DT_{50} of all three test items was calculated to be <24 hours. The DT_{90} which was actually also the DT_{100} was <48 hours.

Conclusion

The three test items α-terpinene, p-cymene and d-limonene disappear rapidly from the soil by evaporation. The DT_{50} of all three test items was calculated to be <24 hours. The DT_{90} which was actually also the DT_{100} was <48 hours.

This study confirms the assumptions made based on the physical chemical properties of the terpenoid blend QRD 460 and the fugacity models conclusions that the fate of the terpenoid blend (α-terpinene, p-cymene and d-limonene) QRD 460 in soil is of limited relevance as it volatilises and evaporates rapidly into the air compartment.

IIA 7.2.2 Aerobic degradation of the active substance in soil at 10°C

Due to its volatility, air is the major environmental compartment of relevance and the degradation of QRD 460 in soil is a minor compartment of concern relative to its fate in the environment. Therefore aerobic degradation at lower temperatures in soil is not considered further.

The results of the soil degradation study at ambient temperature demonstrate that the terpene components of QRD 460 disappear rapidly from the soil by evaporation with a DT90 of <48 hours and so even if the DT90 were to increase with lower temperature, the increase would still result in rapid movement to the atmosphere and degradation would not be expected to take place in the soil. The highly volatile nature of these terpenes confirms this.

IIA 7.2.3 Aerobic degradation of relevant metabolites, degradation and reaction products in soils at 20°C

The aerobic degradation of QRD 460 in soil is a minor compartment of relevance to its fate in the environment as the terpenes have been shown to be very volatile. Due to its rapid dissipation into air there are no relevant metabolites, degradation and reaction products in soils from the use of QRD 460 to be considered further.

IIA 7.2.4 Anaerobic degradation of the active substance in soil

The anaerobic degradation of QRD 460 in soil is a minor compartment of relevance to its fate in the environment as the terpenes have been shown to be very volatile.Therefore anaerobic degradation in soil is not considered further for QRD 460.

IIA 7.2.5 Anaerobic degradation of relevant metabolites, degradation and reaction products in soil

The anaerobic degradation of QRD 460 in soil is a minor compartment of relevance to its fate in the environment as the terpenes have been shown to be very volatile. Due to its rapid dissipation into air anaerobic degradation in soil is not considered further for QRD 460.

IIA 7.3 Field studies

Not considered necessary for an active substance that has been shown to volatize rapidly from soil and primarily degrades in air.

IIA 7.3.1 Soil dissipation testing in a range of representative soils – (normally 4 soils)

See 7.3 above.

IIA 7.3.2 Soil residue testing

See 7.3 above.

IIA 7.3.3 Soil accumulation testing on relevant soils

See 7.3 above.

IIA 7.4 Mobility studies

The Terpenoid blend (α-terpinene, p-cymene, d-limonene) QRD 460 is rapidly volatilised from the surface of the soil and so its mobility in soil does not warrant further consideration.

IIA 7.4.1 Adsorption and desorption of the active substance

See 7.4 above.

IIA 7.4.2 Adsorption and desorption of all relevant metabolites, degradation and reaction products in 3 soils

See 7.4 above.

IIA 7.4.3 Column leaching studies with the active substance

See 7.4 above.

IIA 7.4.4 Column leaching studies with relevant metabolites, degradation and reaction products

See 7.4 above.

IIA 7.4.5 Aged residue column leaching

See 7.4 above.

IIA 7.4.6 Leaching (TLC)

See 7.4 above.

IIA 7.4.7 Lysimeter studies

See 7.4 above.

IIA 7.4.8 Field leaching studies

See 7.4 above.

IIA 7.4.9 Volatility – laboratory studies

Laboratory studies have not been performed because volatility/evaporation from soil is assumed because the physical-chemical properties of the three terpenes in QRD 460 (α-terpinene, p-cymene and d-limonene, indicate high vapour pressures and high Henry's Law Constants. This means that the dominant environmental sink for these compounds is the atmosphere. Monoterpenes, as a class, are released from vegetation in large amounts to the air (Fehsenfeld *et al*. 1992, Guenther *et al.* 1995) which supports the assumption that volatilisation is the most important environmental dissipation pathway for these compounds. Once in the air, research publications and predictive modelling indicate they are degraded relatively rapidly based on interactions with hydroxyl radicals, ozone and nitrate radicals, the latter at night. This is discussed further under Section 7.10 Fate in Air.

IIA 7.5 Hydrolysis rate of relevant metabolites, degradation and reaction products at pH values 4, 7 and 9 under sterile conditions, in the absence of light

The three terpene components in terpenoid blend (α-terpinene, p-cymene and d-limonene) QRD 460 do not contain any functional groups that are susceptible to hydrolysis. Additionally, these three compounds display low water solubility and high vapour pressure, indicating that volatilization is expected to represent a major route of dissipation for these compounds and so it is not necessary to consider hydrolysis further. This is further discussed in Section 1.

IIA 7.6 Direct phototransformation of relevant metabolites, degradation and reaction products in water using artificial light (simulating sunlight and excited wavelengths λ < 290 nm) under sterile conditions

The maximum possible direct photolysis rate constant is zero, resulting in direct photolysis half-lives of infinity for all three in terpenoid blend (α-terpinene, p-cymene and d-limonene) QRD 460. The direct photolysis half-lives of these terpenes, calculated based on the maximum estimated photolysis rate constants, are greater than 30 days, and therefore, according to OECD 316 and OPPTS 835.2210 guidelines, no further direct photolysis work is necessary and so it is not necessary to consider phototransformation further.

IIA 7.7 Ready biodegradability of the active substance

As volatilisation is the most important environmental dissipation route for terpenoid blend (α-terpinene, p-cymene and d-limonene) QRD 460, it is not necessary to consider ready biodegradability of QRD 460 in soil and water further.

IIA 7.8 Degradation in aquatic systems

Introduction

From the fugacity models included in the introduction to this section, it is clear that the active substance, terpenoid blend (α-terpinene, p-cymene and d-limonene) QRD 460 exhibits the main environmental characteristic of dissipating into the air compartment by volatilisation, all three terpene components being extremely volatile in nature.

On this basis, the route and rate of degradation in water will have limited applicability to the environmental fate of QRD 460 when applied as a pesticide. Even when used in the field, the volatile nature of the terpene components will clearly still dominate and this is confirmed by both modelling and a study in natural water as follows.

To ensure as full an assessment as possible, a literature review has been conducted and further modelling considered in the following information summarised below, addressing each of the QRD 460 constituents individually and then this work is compared to the results of a study performed to GLP showing the degradation of the QRD 460 in natural waters, Point 7.8.3.

Fate of α-Terpinene in Water

There are no functional groups such as esters, amides or epoxides in α-terpinene that can hydrolyze. The HYDROWIN program of EPI Suite version 4.0 cannot estimate a hydrolysis rate constant because there are no functional groups that can hydrolyze. The vapor pressure of α-terpinene is high (0.8 mm Hg; 1.06 x 10^2 Pa [15]) and its solubility in water is relatively low giving a high Henry's Law Constant (2.56 x 10^{-2} atm-m^3/mole) which predicts a high rate of volatility from water (EPI Suite version 4.0).

Using the EPI Suite™ model, a river, 1 meter deep with a current velocity of 1 meter/second and a wind velocity of 5 meters/second, the volatilization half life of α-terpinene is predicted to be 1.2 hours. In a lake 1 meter deep with a current velocity of 0.05 meters/second and a wind velocity of 0.5 meters/second, the volatilization half life of α-terpinene is predicted to be 111 hours (4.6 days) from EPI Suite version 4.0.

Although the predictive model provides an idea as to the volatility of α-terpinene from natural waters, an actual water study under static water conditions has shown that 90% of α-terpinene is volatilized within 13.7 hours (see Point 7.8.3).

Fate of p-Cymene in Water

P-Cymene contains no functional groups that can hydrolyze such as esters, amides or epoxides.

The vapor pressure of p-cymene is high (1.46 – 2.0 mm Hg; 1.95 x 10^2 Pa – 2.67 x 10^2 Pa [18, 19]) and its solubility in water is relatively low (23 mg/L) giving a high Henry's Law Constant (1.36 10^{-2} atm-m^3/mole) which predicts a high rate of volatility from water (EPI Suite version 4.0.).

In a river, 1 meter deep and a current velocity of 1 meter/second and a wind velocity of 5 meters/second, the volatilization half life of p-cymene is predicted to be 1.2 hours. In a lake 1 meter deep with a current velocity of 0.05 meters/second and a wind velocity of 0.5 meters/second, the volatilization half life of p-cymene is predicted to be 111 hours (4.6 days [EPI Suite version 4.0]).

Although the predictive model provides an idea as to the volatility of p-cymene from natural waters, an actual water study under static water conditions has shown that 90% of p-cymene is volatilized within 37.4 hours (see Point 7.8.3).

Fate of d-Limonene in Water

There are no functional groups such as esters, amides or epoxides in d-limonene that can hydrolyze. The hydrolytic half life of d-limonene has been estimated to be > 1000 days (Assessment tool for the evaluation of risk, USEPA cited in Hakola, 1994). The vapour pressure of d-limonene is relatively high (1.0 mm Hg; 1.33 x 10^{2} Pa (MSDS 2010) and its solubility in water is relatively low giving a high Henry's Law Constant (1.28 x 10^{-2} atm-m^3/mole) which predicts a high rate of volatility from water.

Using EPI Suite ™ version 4.0, modeling a river, 1 meter deep with a current velocity of 1 meter/second and a wind velocity of 5 meters/second, the volatilization half-life of d-limonene is predicted to be 1.2 hours (EPI Suite ™ version 4.0). In a lake 1 meter deep with a current velocity of 0.05 meters/second and a wind velocity of 0.5 meters/second, the volatilization half life of d-limonene is predicted to be 111 hours (4.6 days).

Although the predictive model provides an idea as to the volatility of d-limonene from natural waters, an actual water study under static water conditions has shown that 90% of d-limonene is volatilized within 10 hours (see Point 7.8.3).

IIA 7.8.1 Aerobic biodegradation in aquatic systems, including identification of breakdown products and metabolites

This is not an EC data requirement.

IIA 7.8.2 Anaerobic biodegradation in aquatic systems, including identification of breakdown products and metabolites

This is not an EC data requirement.

IIA 7.8.3 Water/sediment study

Report:	IIA 7.8.3/01. [redacted], 2011. (R)-(+) Limonene, p-Cymene, α-Terpinene: The Nature and Rate of the Degradation of the Active Components of QRD 460 in Water. [redacted]. Study # 1145.002.254, 04 March 2011.

Guidelines

The methods described in this study plan are not based on a specific guideline but on the expected behaviour of the test item in the environment.

GLP: Yes.

Executive Summary

This study is not a water sediment study, rather a study in natural waters. Degradation of α-terpinene, p-cymene and (R)-(+) limonene (Note: the terms d-limonene and (R)-(+) limonene are synonyms for the same limonene isomer; the terms are equivalent and interchangeable.) was studied in natural lake water. Stock solutions of the three test items were filled into test vessels equipped with traps containing isooctane as trapping solution to collect volatile test item or possible degradation products. A continuous flow-through test system was used at a temperature of 20 ± 2ºC in the dark. Environmental conditions were maintained by continuous aeration. Samples were analysed immediately after application (hour 0) and after 1, 3, 6, 24, 48 and 96 hours. Their respective trapping solutions

were also analysed. Duplicate samples for each test item were analysed at each sampling interval. The water was extracted with hexane containing internal standard. Analysis was by GC-FID.

The three test items α-terpinene, p-cymene and (R)-(+) limonene dissipated rapidly from water by evaporation. The DT_{50} of the test items is <24 hours, and the DT_{90} (or DT_{100}) is determined to be <48hours. It is unlikely that degradation products were formed.

Materials

Test material	α-terpinene	p-cymene	d-limonene
Description:	Not reported	Not reported	Not reported
CAS number	99-86-5	99-87-6	5989-27-5
Lot/Batch #:	812097	812108	810763
Purity:	92.6%	99.1%	95.0%
Stability of test compound:	Not determined	Not determined	Not determined

Application vehicle: Acetone

Water 5 batches of natural locally available filtered lake water

	SW-16-8-10	SW-16-8-10, 18-8-10 [a]	SW-6-9-10	SW-20-9-10	SW-22-9-10 [b]
pH	8.09	7.68	8.28	8.29	8.08
Temperature (°C)	19.7	23.9	22.7	20.5	22.0
Dissolved Oxygen (mg/L)	8.81	9.13	8.56	8.88	6.73
Conductivity (µS/cm)	275	291	290	290	300
Hardness (mg/L as $CaCO_3$)	150	164	142	148	150
Alkalinity (mg/L as $CaCO_3$)	124	128	105	122	115
TOC (mg/L non purgable organic carbon)	9.17	2.25	7.60	6.24	3.10

(a) Mixture of both batches
(b) Only used for preparation of QC samples

Study Design and Methods

Natural filtered locally available lake water was used. The water was filtered through 0.45 µm. The water was characterized for temperature, pH, dissolved oxygen, conductivity, hardness, alkalinity and TOC

Test vessels consisted of photolysis test vessels (volume 20 mL) with screw-cap and covered with aluminium foil to exclude the influence of light. The test vessels were incubated at 20 ± 2°C and the temperature was continuously recorded. 10mL samples of trapping solution were maintained in vials in a water bath kept at 10°C to reduce evaporative losses. The test was performed in a flow-through system. Aerobic conditions were maintained by flushing the system continuously with at approximately 2 mL/min. The air was passed through two traps each containing 10 mL 2,2,4-trimethylpentane (iso-octane) for trapping the volatile test items or their degradation products. To ensure that no test item was lost in the event airflow was stopped, another trap was set before the test vessel. Application solutions for each test item were prepared by dissolving them in acetone and placing 20 mL of application solution into the photolysis vessels. Sampling intervals were 0, 1, 3, 6, 24, 48 and 96 hours after application. Duplicate samples were analysed at each interval.

Water samples were extracted using n-hexane containing internal standard as the solvent. Vials were repeatedly shaken by hand for 20 seconds followed by vortexing for 10 seconds. The whole sample was then transferred into a test tube. The upper phase containing n-hexane was then removed with a pipette. A sub-sample of the extract was then analysed for test item and degradation products using GC-FID. The identification of the metabolites was

performed using GC with mass spectrum (GC-MS). The selection of samples to be analysed with GC-MS was based on the detection of additional peaks in the GC-FID chromatogram not present in the blank samples (internal standard solution). Only metabolites which eluted after the solvent (n-hexane) were recorded on the GC-MS.

DT_{50} and DT_{90} values were determined using a Simple First Order (SFO) kinetic model.

Results and Discussion

Mass Balance

The study was performed with non-radio labelled test material. Therefore, no mass balance can be given.

Extractable Residues

Immediately after application concentrations of (R)-(+) limonene were 0.295 and 0.314 mg a.i./L, corresponding to recoveries of 31.1 and 33.2%. These recoveries are much lower than the recoveries found for the method validation. No explanation can be given for the low recovery but the tendency to dissipate is clear. The level of (R)-(+) limonene in the extracts decreased continuously until it was lower than LOQ of 0.0197 mg a.i./L by 48 hours after application. The test item was also detected in one replicate of the trapping solution 48 hours after application. Repeat measurements were taken at 0 and 24 hours and these showed a recovery of approximately 50% which is close to the results obtained from the method validation. The repeated samples showed a high deviation from the values obtained from the first series and the reason for this deviation is not understood. Therefore the values from the repeat measurements are not used for the calculation of the half-life.

Immediately after application concentrations of p-cymene were 0.778 and 0.848 mg a.i./L, corresponding to recoveries of 78.1 and 85.3%. These recoveries are very similar to the method validation recoveries indicating reliable data despite low recovery. The level of p-cymene in the extracts decreased continuously until it was lower than LOQ of 0.0246 mg a.i./L at 48 hours after application. The test item p-cymene was also detected in the first trapping solution. The amount detected ranged from 0.182 mg as/L (6 hours) to 0.423 mg as/L (96 hours).

Immediately after application concentrations of α-terpinene were 0.472 and 0.465 mg a.i./L, which is equal to recoveries of 46.9 and 46.3%. These recoveries are very similar to the method validation recoveries indicating reliable data despite low recovery. The concentrations decreased continuously. One day after application (hour 24), the level of α-terpinene was already below the LOQ of 0.0657 mg a.i./L. The level in the trapping solutions did not exceed the concentration of the lowest analytical standard (LOQ) at any time point during the study.

Metabolite Identification by GC-MS

No metabolites resulting from the test items were identified.

Degradation Kinetics

Table IIA 7.8.3-1: Degradation rates in water

	DT_{50} (hours)	DT_{90} (hours)	Error level Chi^2 test
α-terpinene	4.1	13.7	19.8
p-cymene	11.2	37.4	23.8
(R)-(+) limonene	3.0	10.0	11.8

Conclusion

The three test items, α-terpinene, p-cymene and d-limonene volatilized from the natural water test systems rapidly with DT_{50}s of 4.1, 11.2 and 3.0 hours and DT_{90}s of 13.7, 37.4 and 10.0 hours for α-terpinene, p-cymene, d-limonene respectively. This means that a DT_{100} could be proposed for QRD 460 of <48 hours. The trapping solutions showed the presence of the test substances but no degradates. Degradates in the water were also not detected. Thus, rapid escape (fugacity via volatility) appears to be the predominant pathway for all three terpenes in natural water.

IIA 7.9 Degradation in the saturated zone of the active substance, metabolites, degradation and reaction products

QRD 460 is rapidly volatilised from water and so its degradation in the saturated zone does not warrant further consideration.

IIA 7.10 Rate and route of degradation in air

Rate of Atmospheric Degradation.

The three terpenes in terpenoid blend (α-terpinene, p-cymene and d-limonene) QRD 460 are degraded quickly in air. Rates of degradation were estimated using the AOPWIN (Atmospheric Oxidation Program for Microsoft Windows program in EPI Suite™ 4.0. The program estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. It also estimates the rate constant for the gas-phase reaction between ozone and olefinic/acetylenic compounds. Finally, it also estimates the rate constant for gas-phase reactions between nitrate radicals and organic chemicals that occur at night. The rate constants estimated are then used to calculate atmospheric half-lives for organic compounds based on average atmospheric concentrations of the hydroxyl radicals, ozone and nitrate radicals ([redacted] 2011).

The estimation methods used in AOPWIN are based on the structure-activity relationship methods developed by Atkinson and co-workers with some updates by EPA contractors. AOPWIN only requires chemical structures to make the estimations. Atkinson's work and the work of his colleagues for estimating half lives of organic chemicals in the atmosphere has been reviewed in Section 3.3 of the Focus Working Group on Pesticides in Air Report (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.).

Table 7.10-1 summarizes the estimated atmospheric half lives of the three terpenes in terpenoid blend (α-terpinene, p-cymene and d-limonene) QRD 460.

Table 7.10-1. Estimated half lives of the monoterpenes in air based on the AOPWIN in EPI Suite™ 4.0.

Compound	Half Life in Air	Reactant
α-terpinene	29.1 minutes 1.7 minutes "may be important"	hydroxyl radicals ozone nitrate radicals
p-cymene	15 hours	hydroxyl radicals
d-limonene	53 minutes 17.3 minutes 0.9 - 9 minutes	hydroxyl radicals ozone nitrate radicals

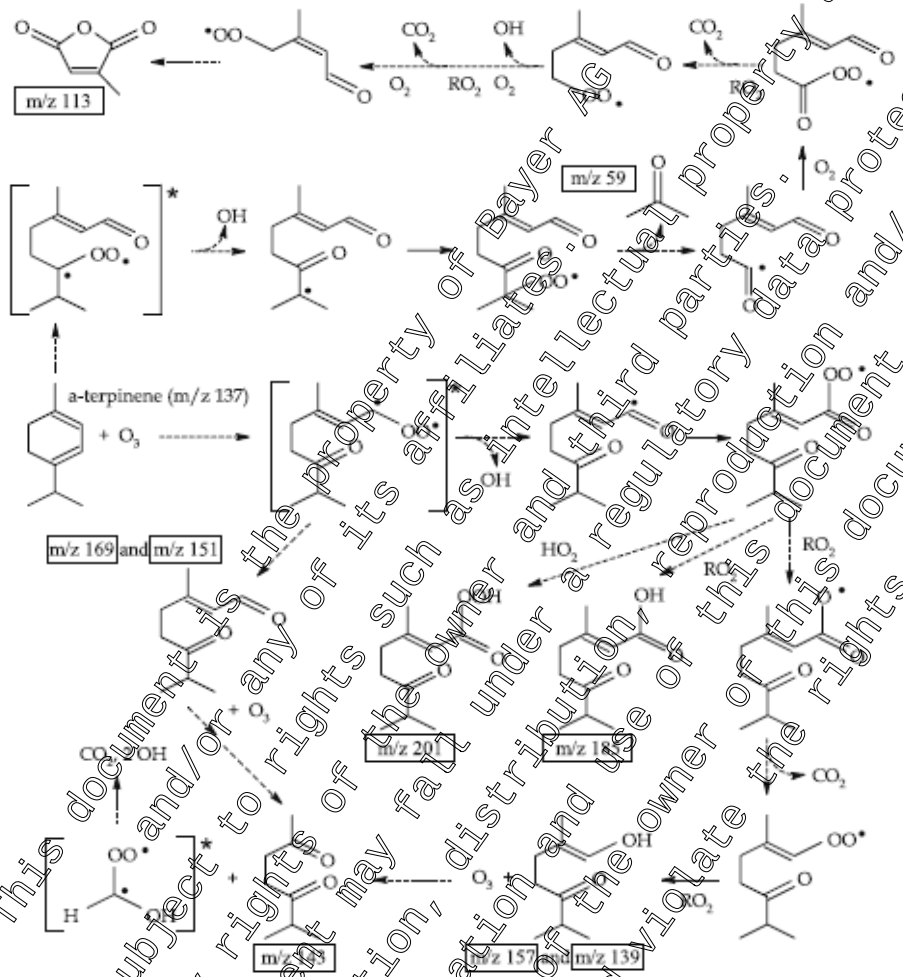
It is appropriate to consider the fate of each terpene individually and so information from a literature search has been summarised as follows.

Route of Atmospheric Degradation of α-Terpinene

Identity and quantification of gas-phase products from the ozonolysis of α-terpinene was reported by Lee *et al.* (2006). This monoterpene was rapidly oxidized (within 30 minutes) with the formation of numerous gas-phase products whose structures were deduced by mass spectrometry. Lower molecular weight products included formaldehyde (4 % molar yield), acetaldehyde (1 % molar yield), formic acid (10% molar yield), acetone (6 % molar yield), acetic acid (10% molar yield) and unidentified products (31 %). Based on the structural assignments

derived from mass spectrometry, a partial mechanism for the ozonolysis of α-terpinene was proposed and is presented in Figure 7.10-1 below.

Figure 7.10-1. Partial mechanism for the ozonolysis of α-terpinene (Lee *et al*, 2006)



The authors noted that the highest yield of a single product, other than the low molecular weight products, accounted for no more than 6 % and that dominant first-generation products were not detected. Thus, certain observed product ions were likely second generation entities. Thus, α-terpinene is readily degraded by ozone in the air to form numerous gas-phase products.

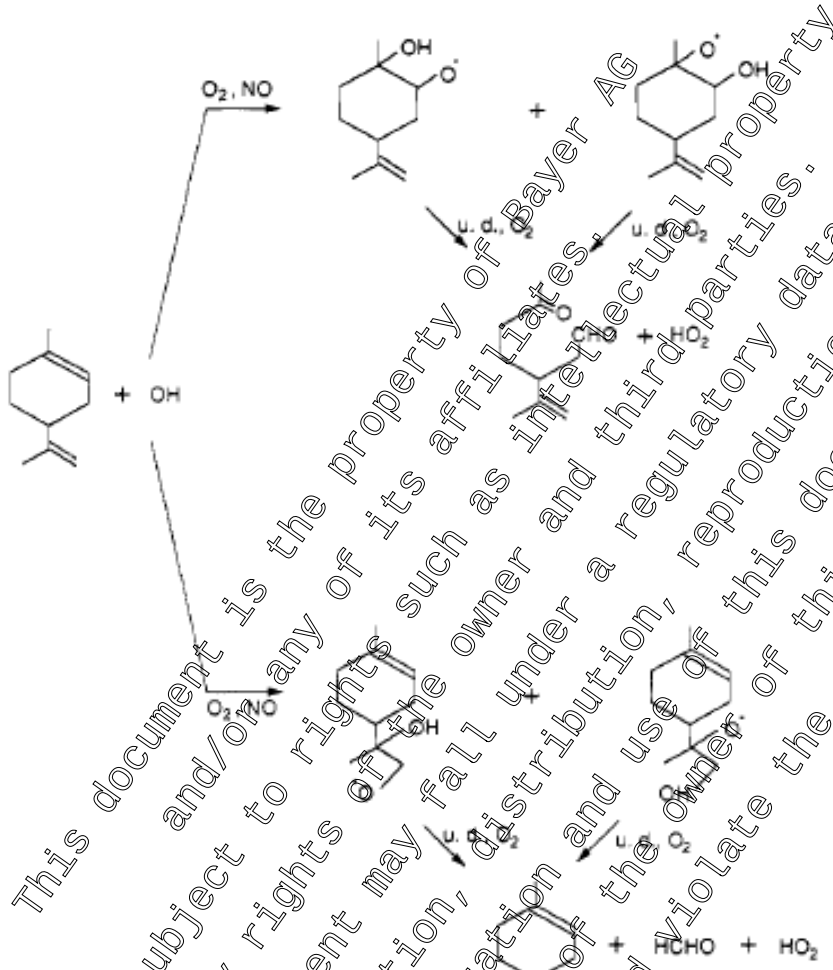
Route of Atmospheric Degradation of p-Cymene

Literature discussing the nature of the degradation of p-cymene in air was not available. Thus, for p-cymene, there are just the estimates for the rate of degradation in air. However all three terpenes are very similar in structure and physical chemical characteristics so it is highly likely that their breakdown in air is similar and certainly rapid.

Route of Atmospheric Degradation of d-Limonene

Grosjean *et al*. (1992) studied the atmospheric oxidation of d-limonene and characterized the reaction products. They are depicted in Figure 7.10-2.

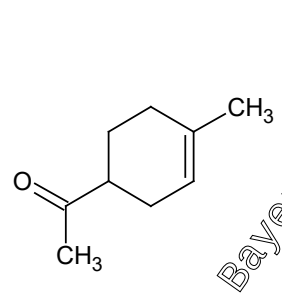
Figure 7.10-2. Reaction products of the d-limonene-hydroxyl radical reaction taken from Figure 2 of Grosjean et al., 1992. The abbreviation u.d. refers to unimolecular decomposition.



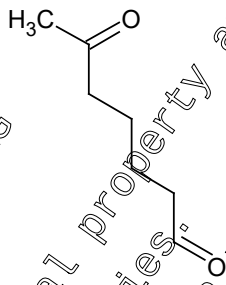
As shown, OH radicals add across either of the two unsaturated carbon-carbon bonds to ultimately form carbonyl degradates.

Hakola *et al*. (1994) also identified 4-acetyl-1-methylcyclohexene and a keto-aldehyde (Figure 7.10-3 below) by GC-FID using an authentic reference standard and by GC-MS and GC-FTIR, respectively, thus confirming the identifications for two of the hydroxyl radical-generated carbonyl degradates reported by Grosjean *et al*. (1992).

Figure 7.10-3. Two identified products of the OH radical reaction with limonene (Hakola *et al*. (1994)) .



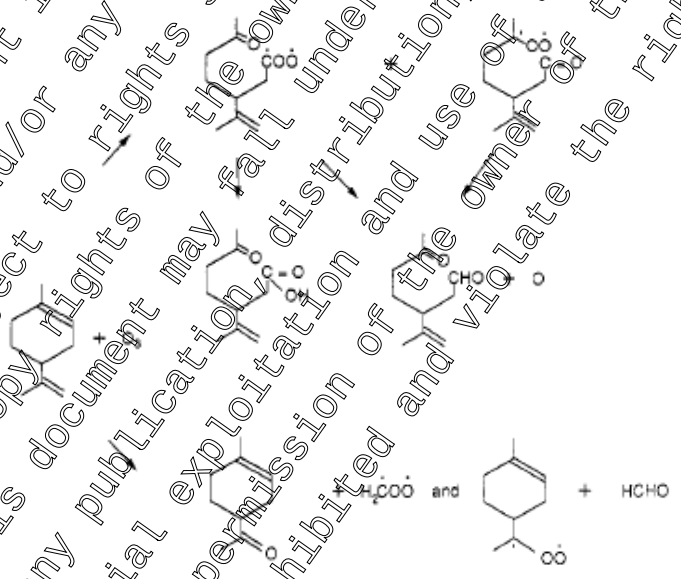
4-acetyl-1-methylcyclohexene



keto-aldehyde

The same carbonyl compounds form (along with formic and C_7 and C_9 carboxylic acids) by reaction of d-limonene with ozone (see Figure 7.10-4 below).

Figure 7.10-4. Simplified mechanism for the interaction of d-limonene with ozone in air (taken from Figure 3 of Grosjean *et al.* (1992) .



Importantly the researchers also noted that the first-generation products were as reactive towards OH radicals and ozone as the parent compound. They go on to mention that the second-generation carbonyl products are not expected to accumulate in the atmosphere but rather undergo rapid oxidation to yield carbon monoxide and free radicals. An example illustrating the further degradation of 4-acetyl-1-methylcyclohexene, formed from the reaction of either hydroxyl radicals or ozone with limonene, is provided in Figure 7.10-5 below. In this case, smaller carbonyl compounds, namely, formaldehyde, glyoxal and 3-oxobutanal, were formed.

Figure 7.10-5. Reactions of 4-acetyl-1-methylcyclohexene with hydroxyl radicals (Grosjean *et al*. 1992).

It is also reported that reactions with oxides of nitrogen produce lower molecular products including formaldehyde, acetaldehyde, formic acid, acetone and peroxyacetylnitrate (International Programme on Chemical Safety, Concise International Chemical Assessment Document No. 5, Limonene, World Health Organization, 1998 (http://www.inchem.org/documents/cicads/cicads/cicad05.htm)).

Thus, reactions of d-limonene with hydroxyl radicals, ozone and nitrate radicals lead to a series of carbonyl compounds that are further converted to very small molecular weight entities.

Conclusions

In conclusion, terpenoid blend (α-terpinene, p-cymene and d-limonene) QRD 460, being highly volatile, is likely to degrade rapidly in air and to form smaller, naturally occurring molecules in the air. This matches the anecdotal evidence from naturally occurring terpenes 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 hours and due to the nature of their chemistry as terpenes, 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 terpenes and from their use as fragrances in household items supports this position.

Risk assessment in Air

Following the principles of the dossier guidelines and the Focus Working Group on Pesticides in Air Report (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.), it is usual to estimate the likely predicted environmental concentration (PEC) of QRD 460 in its product QRD 452. This PEC calculation is usually performed to allow a comparison between the PEC and exposure scenarios in other parts of the dossier. As neither soil or water compartments are viewed as relevant for risk assessment, the following calculation has been performed on the basis that the concentration in glasshouse air is most likely the worst case as it is a technically contained air compartment area (as opposed to the "open air" field).

Calculation of the PEC of the Active Substances in QRD 452 in Glasshouse Air

EU Directive 91/414 requires the calculation of a Predicted Environmental Concentration (PEC) in air although does not provide detailed guidance on how this should be carried out (SANCO/10553/2006 Rev 2 [1]). For QRD 452, a product containing the three terpenes, α-terpinene, p-cymene and d-limonene, the PEC_{air} relevant to a glasshouse application is presented here. The calculation was accomplished as follows:

Assumptions

- ✓ The maximum application rate of QRD 452 in the greenhouse is **1.523 kg** (critical GAP) active substances/ha (10 L product/ha).
- ✓ Area of a typical EU glasshouse is **256 M^2** with a total volume of **901 M^3** (SANCO/10553/2006 Rev 2 [1])
- ✓ All three active substances are volatilized into the glasshouse air immediately after spraying. Previous residue decline studies with tomatoes, mustard greens and primrose at application rates greater than the currently proposed label rates have indicated that the terpenes volatilize within minutes to one hour after spray application (Metabolism and Residues Section 4 [2]). Thus, the assumption of immediate and complete volatilization after spraying represents a reasonable, albeit a worst case, scenario.
- ✓ A glasshouse ventilation rate of **33%/hour** (SANCO/10553/2006 Rev 2 [1])

Thus, 1523 g active substances x 0.0256* = 39 g active substances sprayed
39 g active substances/901 M^3 = 0.043 g/M^3 = 43 mg/1000 L = **0.043 mg/L = $PEC_{greenhouse\ air}$.**

*Area of greenhouse (256 M^2)/area of a hectare (10,000 M^2) = 0.0256 (i.e., 2.56% of a hectare).

It should be noted that all evidence from modelling, the literature and anecdotal evidence suggests that none of the terpene constituents of QRD 460 persist in the air and are rapidly broken down. This means that the PEC air as calculated has limited value as it is a worst case and any exposure is very short lived.

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[1] These studies are also submitted and fully evaluated in Section 4 of the QRD 460 dossier (Points IIA 6.3.1/01, IIA 6.3.3/01 and IIA 6.3.4/01)

[2] 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

Overall Conclusions

The physical-chemical properties of QRD 460 constituents, α-terpinene, p-cymene and d-limonene indicate high vapour pressures and high Henry's Law Constants. This means that the dominant environmental sink for these compounds is likely to be the atmosphere. Monoterpenes, as a class, are released from vegetation in large amounts to the air which supports the assumption that volatilization is the most important environmental dissipation pathway for these compounds. Once in the air, research publications and predictive modelling indicate they are degraded relatively rapidly based on interactions with hydroxyl radicals, ozone and nitrate radicals, the latter at night.

The microbial metabolism (catabolism) of terpenes has been well studied in pure cultures with microbial utilization involving a series of oxidations that provide microbes with both carbon and energy for growth. Specific degradation pathways in microbes have been published. Biodegradation studies using liquid cultures as well as soil also demonstrate rapid assimilation of these terpenes by microbes with concomitant production of biomass and carbon dioxide. Thus, these terpenes will not persist in air (the major environmental sink) or in soil or sludge.

There are no functional groups for these terpenes that could be hydrolysed. However, as stated, high Henry's Law Constants suggest they will escape from either natural waters or from soil water into the air to be quickly degraded, preventing long-range transport. This rapid rate of volatility, from both natural water and soil, was also observed in two recent studies. That is, DT_{50}s in natural water were found to be less than 12 hours for all three terpenes tested individually and DT_{90}s, representing nearly complete removal of the terpenes from water, were found to be no more than 37 hours. In an aerobic soil degradation study, the terpenes were also quickly lost from soil with DT_{50}s less than 24 hours and complete removal from soil was evident within 48 hours. These experimental results therefore are consistent with the high Henry's Law constants of these compounds and the volatility that would be expected.

In summary, α-terpinene, p-cymene and d-limonene in QRD 460 and its product QRD 452 will not persist in the environmental compartments of air, soil and water. Publications from the open literature provide detailed accounts of likely degradation/utilization pathways as well as the extent and rates of degradation. Mackay's multi-media fugacity model (Levels I and III) also provides valuable information concerning the distribution and fate of these monoterpenes in air, soil and water. Finally, experimental results in natural water and aerobic soil support the predictive models as well as research reports in the open literature.

Although calculated and presented here, a PEC air value has limited value for risk assessment as QRD 460 degrades rapidly in air.

IIA 7.11 Definition of the residue

As the three terpenes present in QRD 460 all degrade rapidly in the environment, primarily in the air due to their volatile nature, in a matter of hours, there is no significant residue expected and so no residue is defined and monitoring would be considered unnecessary.

IIA 7.12 Monitoring data concerning fate and behaviour of the active substance and of relevant metabolites, degradation and reaction products

As the three terpenes present in QRD 460 all degrade rapidly in the environment, primarily in the air due to their volatile nature, in a matter of hours, there is no significant residue expected and so no residue is defined and monitoring would be considered unnecessary.

IIA 7.13 Other/special studies

Not relevant.

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
IIA 7.1 Also submitted under IIA 2.10	[redacted]	2011	Fate of d-Limonene, α-Terpinene and p-Cymene in Air, Soil and Water Unpublished Project ID 2011-AQ-2 March 25 2011	Y	AQ
IIA 7.2.1/01 Also submitted under IIA 4.4/01	[redacted]	2010	d-Limonene, p-Cymene, α-Terpinene: Aerobic Rate of Degradation of the Active Components of QRD 460 in Soil. [redacted]. Study # 1145.002.760, 20 December 2010 GLP, Not Published	Y	AQ
IIA 7.8.3/01 Also submitted under IIA 4.5/01	[redacted]	2011	(R)-(+) Limonene, p-Cymene, α-Terpinene: The Nature and Rate of the Degradation of the Active Components of QRD 460 in Water. [redacted]. Study # 1145.002.254 04 March 2011 GLP, Not Published	Y	AQ
IIA 7.10/01 Also submitted under IIA 6.3.1/01	[redacted]	2005	Raw Agricultural Commodity (RAC) Residue Decline of FACIN 25% EC Applied to Tomato. Landis International, Inc, Report No. 44815A001 GLP, Not Published	Y	AQ
IIA 7.10/02 Also submitted under IIA 6.3.3/01	[redacted]	2007	QRD 400/QRD 416: Residue levels of Terpenes in Mustard Greens from a Trial Conducted in California during 2007. SynTech Research, Inc. Report/Study No. 77SRU07R-1 GLP, Not Published	Y	AQ
IIA 7.10/03 Also submitted under IIA 6.3.4/01	[redacted] et al.	2007	Persistence of FACIN 25% EC on Primrose (Primula acaulis) AgraQuest Study No. AQ 07-020 Not Published	Y	AQ