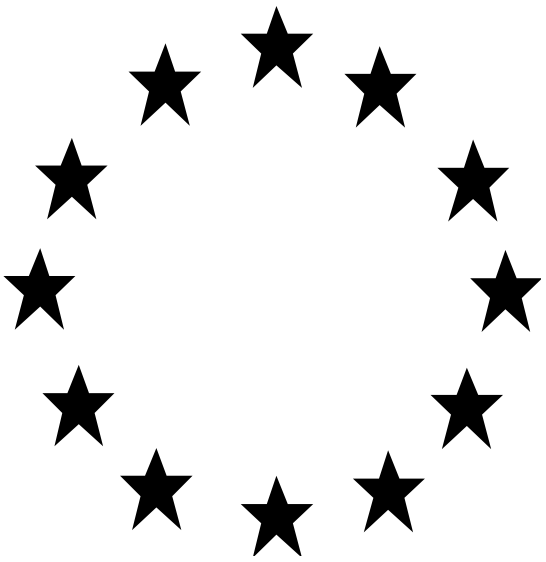


Final addendum to the

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Belgium for the existing active substance

MYCLOBUTANIL

of the third stage Part A of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

January 2009

Table of contents

ANNEX B

Myclobutanil

B.2 Physical and chemical properties (Addendum March 2007)

B.2.1 Physical and chemical properties of the active substance Myclobutanil (Annex IIA 2)

- ***Data requirement 1.1 (cfr. reporting table 1(15)):*** Notifier should provide spectra of relevant impurity 14 (1-methyl-2-pyrrolidinone).
- ***Reporting table, point 1(7):*** DE mentioned an additional estimated value (>3) for the log Pow of myclobutanil.

Table B.2.1-1 : Physical and chemical properties of Myclobutanil

Study	Guidelines/Methods and GLP	Findings	Evaluation and conclusion	References
B.2.1.10 Spectra of the impurities (IIA 2.5.2)	- No guideline referenced (UV/VIS in compliance with OECD 101)	*Impurity 14:* 1-methyl-2-pyrrolidinone (= N-methyl pyrrolidinone; NMP), 99.8% pure (TSN105308; Lot No. 07161HC)) Following spectra were provided: **UV/VIS** (spectra measured between 190-800 nm)	Acceptable The provided spectra for relevant impurity 14 (1-methyl-2-pyrrolidinone) are considered to be acceptable. UV/VIS spectra were recorded	McFarlane, 2005 (Report No. FAPC053382)

<table>
<tr><th>Study</th><th>Guidelines/Methods and GLP</th><th>Findings</th><th>Evaluation and conclusion</th><th>References</th></tr>
<tr><td></td><td>- GLP-compliance stated</td><td>
^{13}C-NMR and ^{1}H-NMR ($CDCl_3$)
MS (EI, positive)
IR (NaCl; sample scanned over range 4000 to 400 cm^{-1})

The different spectra were found to be consistent with the structure of NMP.

UV/VIS absorption characteristics:
<table>
<tr><th></th><th>λ_{max} (nm)</th><th>ε ($L.mol^{-1}.cm^{-1}$)</th></tr>
<tr><td rowspan="2">acidic (pH 0.8)</td><td>203</td><td>4500</td></tr>
<tr><td>at λ 290 nm</td><td><10</td></tr>
<tr><td rowspan="2">Neutral (pH 7.0)</td><td>206</td><td>3400</td></tr>
<tr><td>at λ 290 nm</td><td><10</td></tr>
<tr><td rowspan="2">alkaline (pH 12.6)</td><td>217</td><td>700</td></tr>
<tr><td>at λ 290 nm</td><td><10</td></tr>
</table>
</td><td>and reported according to OECD guideline 101.</td><td></td></tr>
</table>

Study	Guidelines/Methods and GLP	Findings	Evaluation and conclusion	References
B.2.1.13 Partition coefficient n-octanol/water (IIA 2.8)	- Estimation - GLP not relevant (calculation)	During Peer review, an additional estimation of log Pow was suggested: "With the KOWWIN program (v1.67; © 2000 U.S. EPA), a log Pow of 3.5 can be calculated. Moreover, the program's database indicates an experimental log Pow of 2.94 (reference: BioByte, 1995)." RMS has checked these values via website www.syrres.com. Moreover, the RMS considers the log Pow of 2.94, which is mentioned as experimental, to be a calculated estimation as well.	Estimated values of Log Pow are around 3 and hence, bioaccumulation of myclobutanil in the environment is possible. See study Turner (2007) below for an experimental value.	KOWWIN program v1.67; © 2000 U.S. EPA

Study	Guidelines/Methods and GLP	Findings	Evaluation and conclusion	References
	- EEC A8 (Shake flask method) (Determination of myclobutanil concentration in n-octanol and water phase by HPLC-UV) - GLP-compliance stated	*Preliminary estimation (using LOGKOW computer program, version 1.67 U.S. EPA):* Log Pow = 3.50 Experimental determination by 'Shake flask method': Myclobutanil, 99.7% pure (Lot No. F-50-E1662-34); At 20°C: pH 4 buffer solution: log Pow = 3.17 ± 0.01 pH 7 buffer solution: log Pow = 3.17 ± 0.004 pH 9 buffer solution: log Pow = 3.17 ± 0.02 Note: "Since the test substance is surface active, it was recognized that there were limitations to the experimental procedures available for the performance of the test. Consequently during the test, it was ensured that no emulsion formation occurred at the interface of the two liquid phases."	Acceptable According to EEC A8, the shake flask method is not applicable to surface active compounds (myclobutanil is surface active). However, taking into account the fact that special care has been taken during the study with regard to phase separation, the RMS considers the obtained result (i.e. log Pow = 3.17, irrespective of pH) to be reliable. All test conditions were in compliance with those described in EEC A8.	Turner, 2007

B.2.2 Physical, chemical and technical properties of the plant protection product(s) (Annex IIIA 2)

- ***Open point 1.4:*** RMS to include the additional information concerning content of the relevant impurity in the formulation in an addendum or revised DAR.
- ***Data requirement 1.2 (cfr. Reporting table, points 1(10) and 1(20)):*** Shelf life study with composition GF-1317 is required.
- ***Reporting table, point 1(11):*** Additional emulsion stability tests at highest recommended application rate in water A and at lowest recommended application rate (according to doc. D-1: 0.015% v/v) in water A and D are still required.
- ***Reporting table, point 1(12):*** Persistent foaming properties of composition GF-1317 to be tested in water D.

Table B.2.2-1 : Physical, chemical and technical properties of **SYSTHANE 20EW** (Emulsion, oil in water : 200 g/L Myclobutanil)

Study	**Guidelines/Methods and GLP**	**Findings**	**Evaluation and conclusion**	**References**

<table>
<tr><th>Study</th><th>Guidelines/Methods and GLP</th><th>Findings</th><th>Evaluation and conclusion</th><th>References</th></tr>
<tr>
<td>B.2.2.19
Shelf life at ambient temperature
(IIIA 2.7.3)</td>
<td>- GIFAP N° 17
+ methods as indicated under individual points

- GLP-compliance stated</td>
<td>Systhane 20EW (GF-1317), batch C1295-12-B (packed in 1L PET bottle):
<table>
<tr><th></th><th>before storage</th><th>after 2 years at ambient temp</th></tr>
<tr><td>Myclobutanil (g/L) (TM 96-176-02)</td><td>204</td><td>204</td></tr>
<tr><td>appearance</td><td colspan="2">white opaque liquid</td></tr>
<tr><td>density at 20°C (g/mL)</td><td>1.031</td><td>1.031</td></tr>
<tr><td>pH (1% w/v) (MT 75)</td><td>6.33</td><td>8.4</td></tr>
<tr><td>persistent foaming (15.8 g/L in water C) (mL) (MT 47.2)</td><td></td><td></td></tr>
<tr><td>– 10 s</td><td>0</td><td>0</td></tr>
<tr><td>– 1 min</td><td>0</td><td>0</td></tr>
<tr><td>– 3 min</td><td>0</td><td>0</td></tr>
<tr><td>– 12 min</td><td>0</td><td>0</td></tr>
<tr><td>emulsion stability (0.15 g/L and 15.8 g/L in water A and in water D, 30°C) (MT 36)</td><td></td><td></td></tr>
<tr><td>– 30 min</td><td>nil oil/nil cream</td><td>nil oil/nil cream</td></tr>
<tr><td>– 1 hour</td><td>nil oil/nil cream</td><td>nil oil/nil cream</td></tr>
<tr><td>– 2 hours</td><td>nil oil/nil cream</td><td>nil oil/nil cream</td></tr>
<tr><td>– 24 hours</td><td>nil oil/nil cream</td><td>nil oil/nil cream</td></tr>
<tr><td>– 24½ hours</td><td>nil oil/nil cream</td><td>nil oil/nil cream</td></tr>
</table>
</td>
<td>Acceptable
An increase in pH was observed after 2 years of storage, but overall physical parameters were not changed to an extent that would affect product application or safety. Therefore, SYSTHANE 20EW (GF-1317) is considered stable after two years storage in PET bottles at ambient conditions.

Persistent foaming properties were again tested in CIPAC water C (instead of CIPAC water D, as required). For persistent foaming properties tested in water D: see B.2.2.21 (Kendall, 2007; 07-007).</td>
<td>Kendall, 2006
(04-407-G)</td>
</tr>
</table>

Study	Guidelines/Methods and GLP	Findings			Evaluation and conclusion	References
		emulsion stability (5% in <u>water D</u>, 30°C)(MT 36.1.1) – 30 min – 1 hour – 2 hours – 24 hours – 24½ hours	nil oil/nil cream nil oil/nil cream nil oil/nil cream nil oil/nil cream nil oil/nil cream	nil oil/nil cream nil oil/< 0.05% cream nil oil/< 0.05% cream nil oil/< 0.05% cream nil oil/nil cream	*<u>Note:</u> Except for emulsion stability, initial values ('before storage') were taken from GLP study 04-402-G (Tidswell, 2004; ER 60.12).*	
		emulsion stability (5% in <u>water A</u>, 30°C)(MT 36.1.1) – 30 min – 1 hour – 2 hours – 24 hours – 24½ hours	nil oil/nil cream nil oil/nil cream nil oil/nil cream nil oil/0.05% cream nil oil/nil cream	nil oil/nil cream nil oil/nil cream nil oil/nil cream nil oil/< 0.05% cream nil oil/< 0.05% cream		
		particle size (µm) (MT 187) - D(V,0.5) - D(V,0.9)	0.4 1.5	0.3 1.3		
		pourability (MT 148) – % residue – % rinsed residue	5.7 Not determined	4.0 0.2		

Study	Guidelines/Methods and GLP	Findings	Evaluation and conclusion	References
		Statement of notifier with regard to content of relevant impurity NMP in the formulation: *"NMP is present as an impurity in myclobutanil crude and active ingredient. It is not actually produced by any side chemistry in the process, but is only present in the product in small amounts due to the fact that it is the solvent employed in the coupling reaction. The NMP solvent is removed by vacuum distillation and small amounts remain with the product due to the physical difficulty of completely removing the NMP by distillation. Since the NMP impurity is not actually formed in the process due to any side chemistry, it is not possible for the levels of NMP to increase during storage of the active ingredient or any formulations."*	The content of the relevant impurity NMP is unlikely to increase in the formulation upon storage. The notifier's statement is considered to be acceptable.	
B.2.2.21 Persistent foaming (IIIA 2.8.2)	- CIPAC MT 47.2 - No GLP-compliance stated	GF-1317, batch C1295-12-B; Sample taken from filled 1L PET bottle, which had been stored at ambient conditions for approximately 2,5 years: Persistent foaming properties were investigated at a concentration of 15.8 g ppp/L in CIPAC water D: after 10 s : 0 mL foam after 1 min : 0 mL foam after 3 min : 0 mL foam after 12 min : 0 mL foam	Acceptable No persistent foam is expected upon dilution of the preparation GF-1317 with water at the recommended application rates.	Kendall, 2007 (07-007)

Study	Guidelines/Methods and GLP	Findings	Evaluation and conclusion	References
B.2.2.29 Emulsifiability, emulsion stability, re-emulsifiability (IIIA 2.8.7.1)	- CIPAC MT 36.1.1 - GLP-compliance stated	See table under point B.2.2.19: results before storage; Emulsion stability was tested at 0.15 g/L and 15.8 g/L in CIPAC standard waters A and D at 30°C.	Acceptable	Kendall, 2006 (04-407-G)

B.2.3 References relied on

B.2.3.1 Physical and chemical properties of the active substance

Annex Point/ Reference Number	**Author(s)**	**Year**	**Title** **Source (where different from the Company),** **Company, Report Number,** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection claimed (Y/N)**	**Owner**
IIA 2.5.2	McFarlane, J. H.	2005	Determination of the purity and identity of solvent impurity in Myclobutanil Dow AgroSciences LLC, Indiana, USA DAS Report No.: FAPC053382 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
IIA 2.8	Anonymous	-	KOWWIN program (v1.67; © 2000 U.S. EPA) GLP: not relevant Demo version available on internet (www.syrres.com)	N	Not applicable
IIA 2.8	Turner, B.	2007	Determination of octanol/water partition coefficient for Myclobutanil Huntingdon Life Sciences Ltd., Eye, Suffolk, England Project No.: DOS0518/072187 DAS Report No.: NAFST-06-170 GLP: Yes Not published	Y	DAS
IIIA 2.7.3, IIIA 2.8.7.1	Kendall, P.	2006	Systhane 20 EW Fungicide (GF-1317, 200 g/L Myclobutanil) Two years ambient shelf life stability in and compatibility with PET bottle packaging. Dow AgroSciences (NZ) Ltd. DAS Report No. : 04-407-G GLP: Yes Not published	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
IIIA 2.8.2	Kendall, P.	2007	Systhane 20 EW Fungicide (GF-1317, 200 g/L Myclobutanil) Persistent foam performance after two years ambient storage in PET bottle packaging Dow AgroSciences (NZ) Ltd. DAS Report No.: 07-007 GLP: No Not published	Y	DAS

ANNEX B

Myclobutanil

Amended addendum post Praper 19

B.6 Toxicology and metabolism

ADDENDUM

A new package of acute toxicity studies have been conducted on the active substance myclobutanil, obtained from KemFine (batch n° TSN105153; purity 99.7%).

B.6.2 Acute toxicity including irritancy and skin sensitization (Annex IIA 5.2)

B.6.2.1 Acute oral toxicity (Annex IIA 5.2.1)

- **Acute oral toxicity study in rats, up and down procedure (Moore, 2005a)**

Findings:
175 mg/kg and 550 mg/kg dose levels: both animals survived, gain weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse clinical signs, or abnormal behavior. No gross abnormalities were noted for either of the animals when necropsied at the conclusion of the 14-day observation period.
1750 mg/kg dose level: all animals survived to the test substance and gained body weight during the study. Clinical signs observed for 2 rats included ano-genital staining and/or hypoactivity. Animals recovered by day 3. no gross abnormalities were seen.
5000 mg/kg dose level: all rats died within one day. Prior to death, rats werer hypoactive and/or exhibit abnormal posture, ano-genital staining, piloerection and diarrhea. Gross necropsy of the decedents revealed discoloration of the intestines.

Conclusion: LD50=3.129 mg/kg bw in female rats

GLPstatus: yes.
Guideline: study not fully in compliance with OECD guideline 425 (2001)
Deviation from official protocol: one female at 5000 mg/kg bw died: there was no experimental reason to start with a high dose level of 5000 mg/kg bw as mortality was reported at doses < 2000 mg/kg bw in the original dossier. the doses reported in the summary are different from those reported in the study.
Material and methods: 9 Fisher 344 rat received by gavage, a single oral dose of myclobutanil ref n° TSN 105153, 050610-2D; 99.7%)as a 25% suspension in 1..5% solution of carboxymethylcellulose. Rats were fasted overnight. One animal received a dose of 5000 mg/kg and died.
3175 mg/kg bw was the next starting dose. 8 additional females were dosed at levels of 550, 1750 or 5000 mg/kg. females were tested because they are more sensitive to the toxicity of test compounds.
The study is acceptable.

B.6.2.2 Acute percutaneous toxicity (Annex IIA 5.2.2)

- **Acute dermal study in rats- limit test (Moore, 2005b)**

Findings:
All animals survived, gained weight, and appeared active and healthy during the study. There were no signs of gross toxicity, dermal irritation, adverse clinical signs or abnormal behavior.

Conclusion: LD50 dermal > 5000 mg/kg bw

GLPstatus: yes
Guideline: study is conforming to dir EEC 92/69 Annex V- Limit test at 5000 mg/kg bw/d.
Material and methods: 5 Fisher 344 rats/sex received after fur clipping, a patch of myclobutanil (ref n° TSN 105153, 050610-2D; 99.7%) at 5000 mg/kg bw which was mixed with water. The gauze pad and entire trunk were wrapped with tape for 24 hr. The application site was then wiped gently but the substance remained on the skin.
The study is acceptable.

B.6.2.3 Acute inhalation toxicity (Annex IIA 5.2.3)

No new study provided.

B.6.2.4 Skin irritation (Annex IIA 5.2.4)

- **Skin irritation study in rabbits (Moore, 2005c)**

Findings:

The test substance should be considered non-irritating to the skin of rabbits.

<Score erythema>$_{24+48+72h}$=0.666/0/0.333
<score oedema >$_{24+48+72h}$=0.666/0/0.333

Conclusion: myclobutanil is non-irritating to the skin under these experimental conditions.

GLPstatus: yes
Guideline: study is not fully conforming to dir EEC 92/69 Annex V, method B.4
Deviation from official protocol: individual skin scores for erythema are reported together with scores for edema. The use of a lower dose should be clarified.
Material and methods: 1 male and 2 female New Zealand white rabbits received after fur clipping, a patch of myclobutanil (ref n° TSN 105153, 050610-2D; 99.7%)at 770 mg applied as a dry paste (65% mixture in distilled water). The application site was covered for 4 hr <ith semi-occlusive tape. The application site was then wiped gently.
The study is acceptable if the lower dose use is clarified.

B.6.2.5 Eye Irritation (Annex IIA 5.2.5)

- **Eye irritation study in rabbits (Merkel, 2005)**

Findings:

<Score cornea >$_{24+48+72h}$ = 0.666/0/0
<Score iris>$_{24+48+72h}$ = 1/0/0.333
<Score conjuntivae redness>$_{24+48+72h}$ = 1/1/1
<Score conjuntivae chemosis>$_{24+48+72h}$ = 0.333/0.33/0.333

Complete reversibility was reported at 72 h.

Conclusion: myclobutanil is non- irritating to eyes under these experimental conditions.

GLPstatus: yes
Guideline: study not fully in compliance with dir EEC 92/69 method B.5.
Deviation from official protocol: 0.04 g was applied instead of 0.1g.
Material and methods: 3 male New Zealand white rabbits received 0.04 g myclobutanil as a solid test substance (batch n° TSN 105153; 99.7%). One day prior dosing, the eyes were grossly examined and a drop of 2% sodium fluorescein was instilled onto the eye and eyes were flushed with water.
The study is acceptable if the use of a lower dose is justified.

B.6.2.6 Skin sensitization (Annex IIA 5.2.6)

- **Mouse LLNA (Woolhiser et al, 2005)**

Findings:
2 daily topical applications of 1%, 5%, 10%, 20%, 40%, or 80% myclobutanil were given to one animal at each dose level. Erythema was absent in the mice treated with 1%, 5%, and 10% while mice treated with 20%, 40% and 80% showed slight erythema. Body weight of animals was not affected.
In the main test, erythema was absent in the mice treated with 5% and 20% myclobutanil, while5/6 mice treated with 80% myclobutanil showed slight erythema on day 6. Body weight was unaffected. Topical application of 5%, 20% or 80% myclobutanil elicited proliferative responses/stimulation indexes that were respectively 1.1-1.5- and 1.6 fold greater than vehicle controls. Positive control showed the expected response (table B.6.2.6-1).

Table B.6.2.6-1: stimulation index after exposure to myclobutanil

Dose	**Stimulation index**
Myclobutanil	
0%	1.0±0.2
5%	1.1±0.2
20%	1.5±0.6
80%	1.6±0.3

HCA 30%	5.3±2

Conclusion: myclobutanil did not demonstrate dermal sensitization potential in the mouse LLNA.

GLPstatus: yes
Guideline: study in compliance with EC test guideline B.42 (2004) and OECD 429 guideline (2002).
Material and methods:
6 female BALB/cAnNCrl female mice/dose were exposed to 25 µl myclobutanil (batch n° TSN 105153; 99.7%) on days 1-3 at 5%, 20% or 80%. DMSO was used as negative control. On day 6, uptake of 3-thymidine was measured 5 hours post-administration. Positive control was 30% alpha hexylcinnamaldehyde. A screening test was performed in order to evaluate the doses to be used in the main test.
The study is acceptable.

B.6.2.7 Summary of acute toxicity including irritancy and skin sensitization (Annex IIA 5.2)

The company provided a new package of acute toxicity studies from the actual registered source in Brazil. Based on these data, it appears that myclobutanil is of low acute oral and dermal toxicity. It is not irritating to eyes or skin and is not a skin sensitizer under these experimental conditions.

In the original DAR 2 studies for acute oral toxicity were provided. From the first oral study, myclobutanil appeared to be harmful toward male rats and was classified Xn, R22. In the second study, quite comparable results of acute oral toxicity were seen in male and female rats and the obtained results confirming the need for classification as harmful when swallowed. In the eye irritation test, myclobutanil induced corneal vascularization in 1/9 rabbits. These studies were performed with myclobutanil of lower purity than that used here: 91.9% in the first oral study and eye irritation test, and 84.5% in the second oral study.

In the original dossier, a proposed minimum purity of 925 g/kg was accepted taking into account the GLP batch analysis and the purity range of toxicology batches. In the original DAR, some studies such as acute toxicity studies, reproduction and developmental studies, and some genotoxicity studies, were performed with pilot plant batches with a purity of 84.5%. Some chronic studies were performed with a compound of higher purity (90-92%) coming from the final manufacturing process. The purity differed between the two processes as a result of an additional purification step in the final manufacturing process.
The company provided recently a new package of acute toxicity studies in response to Brazilian authorities using a compound of 95.1% produced by KemFine. Except purity, no other information is provided about the origin of the compound.
Therefore, before to take the results of this new package into account, further information should be provided in order to assess the equivalence of the two sources of technical materials. Evaluation of points 1.1-1.11 and 4.1 of Annex IIA of the Directive 91/414/EEC should be performed.

RMS proposes not to take this new package into account as the results of acute toxicity obtained with this new source present a lesser hazard compared to the reference source.

It is RMS opinion that the high increase in purity (from 84% up to 95.1%) could affect the complete toxicology profile of the active ingredient and acute toxicity studies are not sufficient to address the hazard of myclobutanil taking into account the reproduction/developmental toxicity profile of this compound.

Further assessment of equivalence is considered necessary before to change the classification of myclobutanil.

Table B.6.2.7-1: Summary of acute toxicity of myclobutanil

Type of test Test species	**Test substance purity**	**Results**	**References**
Acute oral rat	Batch n° TSN105153;99	LD50females=3129mg/kg bw	Moore, 2005
Acute, dermal, rat	Batch n° TSN105153;99	> 5000 mg/kg bw	Moore, 2005
Rabbit, skin irrita	Batch n° TSN105153;99	Not irritating	Moore, 2005
Rabbit, eye irritati	Batch n° TSN105153;99	Not irritating	Merkel, 2005

Mouse local LNA	Batch n° TSN105153;99	Not sensitizer	Woolhiser et al., 2005

B.6.3 Short term toxicity (Annex IIA 5.3)

Position Document from the company in response to EFSA/Member State comments (Reporting Table) on the Myclobutanil DAR.

Notifier comments are reported here:

1) Effects in dog livers:

Comments from the Reporting Table:

90-Day dog study: 2(7) NL: The effects on the liver cannot be regarded as 'just' adaptive. The high increase in liver weight (varies from 9%-52%) in combination with the histopathology (centrilobular/midzonal hepatocyte hypertrophy) is definitely an adverse effect. For the females, the NOAEL is 200 ppm (7.88 mg/kg bw/d) and for the males 10 ppm (0.34 mg/kg bw/d).

1-Year dog study: 2(8) NL: The high increase in liver weight of 27% at 400 ppm in combination with the histopathology (hypertrophy) in 2 animals is an adverse effect. The NOAEL for this study is 100 ppm (3 mg/kg bw/d).

2(10) DE: Remark: The liver is clearly the target organ. Therefore, the NOAEL in the 90-day study in dogs is seen at 10 ppm based on concomitant relative liver weight increase and hepatocyte hypertrophy at 200 ppm. Similar effects were noted in the 1-yr study at 400 ppm with the next lower dose of 100 ppm being a clear NOAEL. Thus, 100 ppm (ca 3 mg/kg bw/d) can be considered an overall NOAEL for subchronic toxicity in dogs. Liver effects in dogs should be discussed on an EPCO meeting.

2(25) NL: If the NOAEL in the dog studies will be reconsidered based on the NL comments (see comments 1 and 2), the 'overall' NOAEL of the dog studies will be 3 mg/kg bw/d. The AOEL will then be 0.03 mg/kg bw/d.

2(26) DE: Proposal: A lower AOEL of 0.03 mg/kg bw/d is proposed that should be derived from the suggested overall NOAEL for subchronic toxicity in dogs (see comment above). Discussion on an EPCO meeting is recommended.

2(28) UK: Due to the magnitude of the liver weight effects in females at 400 ppm in the 1 year dog study, combined with the increased SAP activity and histopathology, the UK considers that this study derives a NOAEL of 100 ppm. This is lower than that obtained in the rat multigeneration study, and should be used in the derivation of the AOEL.

The company summarized the effects reported in the dog studies (table B.6.3-1 and table B.6.3-2).

Table B.6.3.-1 90-Day Dog study: Summary of liver effects: NOAEL = 1600ppm

	Males					**Females**				
Dose (ppm)	**0**	**10**	**200**	**800**	**1600**	**0**	**10**	**200**	**800**	**1600**
Dosage (mg/kg bw/day)	0	0.34	7.26	29.13	56.8	0	0.42	7.88	32.43	57.97
Parameter										
SAP (% change) (=ALP)	-	-	-	26	47	-	-	38.6	63.9	246
ALT (% change)	-	-30	-20.7	-12.8	-17.2	-	-17	-30.9	-23.4	-35.8
AST (% change)	-	1.3	17.8	15.6	16.9	-	3.9	-16.7	-7.0	-18.6
Rel Liver wt (%	-	-6.0	9	24	41	-	-9.8	-1.4	12	31

	Males					Females				
Dose (ppm)	**0**	**10**	**200**	**800**	**1600**	**0**	**10**	**200**	**800**	**1600**
increase)										
Centrilobular hypertrophy (incidence)	-	-	3/4*	4/4*	4/4*	-	-	-	4/4*	4/4*

*minimal-mild

Table B.6.3-2: 1-Year Dog study: Summary of liver effects: NOAEL = 400 ppm

	Males					Females				
Dose (ppm)	**0**	**10**	**100**	**400**	**1600**	**0**	**10**	**100**	**400**	**1600**
Dosage (mg/kg bw/day)	0	0.34	3.09	14.28	54.22	0	0.40	3.83	15.68	58.20
Parameter										
SAP (% change) (ALP) – Week 13	-	6.9	-13.4	14.2	59.6	-	23.3	41.6	41.6	196
Week 53	-			33	143				59.5	225
ALT (% change) – Week 13	-	-	2.0	8.6	11.1	-	-	-1.7	-23.7	-6.0
Week 25	-	-	4.0	-18.4	36.8	-	-	0	-8.1	6.0
Week 39	-	-	-3.8	-20.5	22.4	-	-	4.6	-8.6	20.8
Week 53	-	-	-2.1	-13.5	23.6	-	-	-8.4	-13.2	11.5
AST (% change) – Week 13	-	-3.2	-8.1	3.6	-8.1	-	1.1	-10.7	-8.8	-5.5
Week 53	-	6.6	3.9	4.4	6.1	-	4.4	-2.2	-4.0	-4.4
Rel Liver wt (% increase)	-	1.7	-0.3	14.2	43	-	19.3	13.8	27	52
Centrilobular hypertrophy (incidence)	-	-	-	1/6*	5/6*	-	-	-	2/6**	6/6**
Ballooned hepatocytes	-	-	-	-	-	-	-	-	-	4/6

*minimum to mild
**mild to moderate

The EPA HED Guidance Document (#G0201) on Hepatocellular Hypertrophy suggests a weight-of-evidence approach to assessing the relevance of liver changes associated with hepatocellular hypertrophy and liver weight increases. Increases in ALT/AST should be 2-3 folds higher than controls in order to be considering as potentially adverse. Increased ALT alone may be due to an isozyme of extra-hepatic origin. Thus, serum levels of at least 2 clinical chemistry parameters should be significantly elevated to ascribe an adverse effect in the liver, for both subchronic and chronic effects. Also, the severity of the hypertrophy alone does not indicate an adverse effect.

In the studies with myclobutanil, using this weight of evidence approach, it is clear that in the 90-day study, the NOAEL is 1600 ppm as the hypertrophy (minimal to mild severity) is accompanied by liver weight increases, with no other related parameters affected. There is no adverse effect on ALT. There is no effect on AST in either the 90-day or 1-year study. The changes are considered to be adaptive, and not adverse in the absence of further changes related to degeneration. Increases in ALP are observed which may be indicative of enzyme induction, and thus support the fact that the liver is showing adaptive changes to exposure (2-fold change only observed in high dose females at 90-days).

Based on the data in the 1-year study, the NOAEL is considered to be 400 ppm, again due to the minimal-mild hypertrophy, accompanied by liver weight increases. Changes in ALT are minimal and do not worsen with increased exposure duration. The 1600 ppm dose group is considered a LOAEL due to the ballooning of the hepatocytes for females. Changes in ALP were again observed (2-fold at the high dose), indicative of enzyme induction, and did not worsen with increased duration of exposure. Effects seen at the 13-week stage of the study were comparable to those seen in the 90-day study. At the 13-week sampling point, there are no adverse effects on clinical chemistry related to liver toxicity.

The overall NOAEL in the dog can therefore be considered to be 14.28 mg/kg bw/day.

This conclusion is in agreement with RMS proposal in the DAR.

However, during Praper 19, March 2007, the experts consider that liver hypertrophy is an adverse effect and should therefore be taken into account for setting of NOAELs.
The 2 dog studies were re-examined and it was concluded that the NOAEL for the 90-day dog study should be set at 10 ppm and the NOAEL for the 1 year dog at 100 ppm.
An overall NOAEL for the dog studies is proposed at 100 ppm and should be used for setting of AOEL.

Recently published studies from open literature related to the mode of action of conazoles:

In 2006, studies were published in the open literature trying to understanding the basis of species differences in conazole carcinogenesis combining transcriptional and toxicological approaches.
Due to limitation in time, it was not possible to RMS to do a complete evaluation of the different published papers. However, the abstracts of the different papers are reported here and some conclusions are proposed.

The following papers were published in 2006:

- Toxicity profiles in rats treated with tumorigenic and nontumorigenic triazole conazole fungicides: Propiconazole, triadimefon, and myclobutanil (Wolf et al., Toxicol. Pathol 2006;34(7):895-902)

The present study was designed to identify commonalities of effects across the different conazoles and to determine unique features of the tissue responses that suggest a toxicity pathway and a mode of action for the observed thyroid response for triadimefon. **Male Wistar/Han rats were treated with** triadimefon (100, 500, 1800 ppm), propiconazole (100, 500, 2500 ppm), or **myclobutanil (100, 500, 2000 ppm) in feed for 4, 30, or 90 days**. The rats were evaluated for clinical signs, body and liver weight, histopathology of thyroid and liver, hepatic metabolizing enzyme activity, and serum T3, T4, TSH, and cholesterol levels. There was a dose-dependent increase in liver weight but not body weight for all treatments. The indication of cytochrome **induction, pentoxyresorufin O-dealkylation (PROD) activity**, had a dose-related increase at all time points for all conazoles. Uridine diphopho-glucuronosyl transferase **(UDPGT**), the T4 metabolizing enzyme measured as glucuronidation of 1-naphthol, was **induced** to the same extent after 30 and 90 days for all three conazoles. Livers from all high dose treated rats had **centrilobular hepatocyte hypertrophy** after 4 days, while only triadimefon and propiconazole treated rats had hepatocyte hypertrophy after 30 days, and only triadimefon treated rats had hepatocyte hypertrophy after 90 days. Thyroid follicular cell hypertrophy, increased follicular cell proliferation, and colloid depletion were present only after 30 days in rats treated with the high dose of triadimefon. A dose-dependent decrease in T4 was present after 4 days with all 3 compounds but only the high doses of propiconazole and triadimefon produced decreased T4 after 30 days. T3 was decreased after high-dose triadimefon after 4 days and in a dose-dependent manner for all compounds after 30 days. Thyroid hormone levels did not differ from control values after 90 days and TSH was not increased in any exposure group. A unique pattern of toxic responses was not identified for each conazole and the hypothesized mode of action for triadimefon-induced thyroid gland tumors was not supported by the data.

- Transcriptional profiles in liver from rats treated with tumorigenic and non-tumorigenic triazole conazole fungicides: Propiconazole, triadimefon, and myclobutanil (Hester et al., Toxicol Pathol. 2006;34(7):879-894)

The goal of the present study was to define pathways that explain the biologic outcomes reported in the previous paper. Male Wistar/Han rats (3 per group), were exposed to the 3 conazoles in the feed for 4, 30, or 90 days of treatment at the same doses as reported in the previous paper. Hepatic gene expression was determined using high-density Affymetrix GeneChips (Rat 230_2). Differential gene expression was assessed at the probe level using Robust Multichip Average analysis. Principal component analysis by treatment and time showed within group sample similarity and that the treatment groups were distinct from each other.

The number of altered genes varied by treatment, dose, and time. The greatest number of altered genes was induced by triadimefon and propiconazole after 90 days of treatment, while myclobutanil had minimal effects at that time point.
Pathway level analyses revealed that after 90 days of treatment the most significant numbers of altered pathways were related to cell signaling, growth, and metabolism. Pathway level analysis for triadimefon and propiconazole resulted in 71 altered pathways common to both chemicals. These pathways controlled cholesterol metabolism, activation of nuclear receptors, and N-ras and K-ras signaling. There were 37 pathways uniquely changed by propiconazole, and triadimefon uniquely altered 34 pathways.
Pathway level analysis of altered gene expression resulted in a more complete description of the associated toxicological effects that can distinguish triadimefon from propiconazole and myclobutanil.

Conclusion of RMS: the results of the study show that at 90 day, myclobutanil treatment did not produce substantial numbers of significant changed genes involved in cell signaling, growth and differentiation. Triadimefon and propiconazole altered more pathways functionally characterized as cell signaling, and growth and differentiation pathways compared to myclobutanil.
Disregulation of cell cycle and metabolic growth processes does not represent key event in myclobutanil hepatotoxicity.

- Toxicity profiles in mice treated with hepatotumorigenic and non-hepatotumorigenic triazole conazole fungicides: Propiconazole, triadimefon, and myclobutanil. (Allen et al., Toxicol Pathol. 2006;34(7):853-62)

Certain conazoles are tumorigenic in rodents; both propiconazole and triadimefon are hepatotoxic and hepatotumorigenic in mice, while myclobutanil is not a mouse liver tumorigen. As a component of a large-scale study aimed at determining the mode(s) of action for tumorigenic conazoles, we report the results from comparative evaluations of liver and body weights, liver histopathology, cell proliferation, cytochrome P450 (CYP) activity, and serum cholesterol, high-density lipoprotein and triglyceride levels after exposure to propiconazole, triadimefon, and myclobutanil. **Male CD-1 mice were treated in the feed for 4, 30, or 90** days with triadimefon (0, 100, 500, or 1800 ppm), propiconazole (0, 100, 500, or 2500 ppm) or **myclobutanil (0, 100, 500, or 2000 ppm**).
Alkoxyresorufin O-dealkylation (AROD) assays indicated that all 3 chemicals induced similar patterns of dose-related increases in metabolizing enzyme activity. **PROD activities exceeded those of MROD**, and EROD with propiconazole inducing the highest activities of PROD. Mice had similar patterns of dose-dependent increases in **hepatocyte hypertrophy** after exposure to the 3 conazoles. High-dose exposures to propiconazole and myclobutanil, but not triadimefon, were associated with early (4 days) **increases in cell proliferation**. All the chemicals at high doses **reduced serum cholesterol and high-density lipoprotein (HDL**) levels at 30 days of treatment, while only triadimefon had this effect at 4 days of treatment and only myclobutanil and propiconazole at 90 days of treatment. Overall, the tumorigenic and nontumorigenic conazoles induced similar effects on mouse liver CYP enzyme activities and pathology. There was no specific pattern of tissue responses that could consistently be used to differentiate the tumorigenic conazoles, propiconazole, and triadimefon, from the nontumorigenic myclobutanil. These findings serve to anchor other transcriptional profiling studies aimed at probing differences in key events and modes of action for tumorigenic and nontumorigenic conazoles.

In this study, the 3 conazoles induce hepatomegaly, induce high levels of PROD activity, increase cell proliferation in liver and decrease serum cholesterol levels and increased triglycerides at 30 days.

-Transcriptional profiles in liver from mice treated with hepatotumorigenic and nonhepatotumorigenic triazole conazole fungicides: propiconazole, triadimefon and myclobutanil (Ward et al., Toxicol. Pathol, 34, 863-878, 2006)

The present study relates the toxicological effects observed in the previous study to alterations of gene and pathway transcription and identifies potential modes of tumorigenic action. In a companion study employing conventional toxicological bioassays (Allen et al., 2006), male CD-1 mice were fed triadimefon, propiconazole, or myclobutanil in a continuous oral-dose regimen for 4, 30, or 90 days. These conazoles were found to induce hepatomegaly, to induce high levels of hepatic pentoxyresorufin-O-dealkylase activity, to increase hepatic cell proliferation, to decrease serum cholesterol, and to increase serum triglycerides.

Differentially expressed genes and pathways were identified using Affymetrix GeneChips. Gene-pathway associations were obtained from the Kyoto Encyclopedia of Genes and Genomes, Biocarta, and MetaCore compendia.
The pathway profiles of each conazole were different at each time point.
In general, the number of altered metabolism, signaling, and growth pathways increased with time and dose and were greatest with propiconazole.
All conazoles had effects on nuclear receptors as evidenced by increased expression and enzymatic activities of a series of related cytochrome P450s (CYP).
A subset of altered genes and pathways distinguished the three conazoles from each other. Triadimefon and propiconazole both altered apoptosis, cell cycle, adherens junction, calcium signaling, and EGFR signaling pathways. Triadimefon produced greater changes in cholesterol biosynthesis and retinoic acid metabolism genes and in selected signaling pathways. Propiconazole had greater effects on genes responding to oxidative stress and on the IGF/P13K/AKt/PTEN/mTor and Wnt-beta-catenin pathways.
In conclusion, while triadimefon, propiconazole, and myclobutanil had similar effects in mouse liver on hepatomegaly, histology, CYP activities, cell proliferation, and serum cholesterol, genomic analyses revealed **major differences in their gene expression profiles**.
Differentially expressed genes and gene expression dose response: the greatest change of increased gene expression after conazole treatment was the xenobiotic metabolizing P450 genes coding for Cyp2b20, Cyp2c55 and Cyp2c65. Cyp 2b20 is a Phenobarbital inducible monooxygenase related to CAR. Cyp2c55 is a recently discovered monooxygenase that metabolizes arachidonic acid and linoleic acid. Cyp 2c65 function is not yet determined.
Myclobutanil did not up regulate cholesterol biosynthetic genes.
Myclobutanil altered some genes in pathways of GSH metabolism, lipid metabolism, cell growth and cell death and membrane transporters. However, the number of genes altered in these pathways were lowest for myclobutanil.
The increased mouse liver weight coupled with the increased Cyp2b20 gene expression at the 3 time points are consistent with a CAR mediated hepatic hypertrophy.

- Gene expression profiling in the liver of CD-1 mice to characterize the hepatotoxicity of triazole fungicides (Goetz et al., toxicol Applied Pharmacol, 215, 2006, 274-284)

Fluconazole, myclobutanil, propiconazole, or triadimefon were were examined for hepatotoxic effects in mouse liver. Besides organ weight, histopathology, and cytochrome P450 (CYP) enzyme induction, DNA microarrays were used to generate gene expression profiles and hypotheses on potential mechanisms of action for this class of chemicals. Adult male CD-1 mice were exposed daily for 14 days at 10, 75 or 150 mg/kg bw/d (myclobutanil) dose levels by oral gavage. Doses were based on previous studies that resulted in liver hypertrophy or hepatotoxicity.
All four triazoles caused hepatocyte hypertrophy, and all except triadimefon increased relative liver/body weight ratios at the middle and high dose levels. CYP enzyme activities were also induced by all four triazoles at the middle and high doses as measured by the dealkylations of four alkoxyresorufins, although some differences in substrate specificity were observed.
Consistent with this common histopathology and biochemistry, several **CYP and xenobiotic metabolizing enzyme (XME) genes were differentially expressed in response to all four (Cyp2d26 and Cyp3a11), or three of the four (Cyp2c40, Cyp2c55, Ces2, Slco1a4) triazoles.** Differential expression of numerous other CYP and XME genes discriminated between the various triazoles, consistent with differences in CYP enzyme activities, and indicative of possible differences in mechanisms of hepatotoxicity or dose response.
Multiple isoforms of Cyp1a, 2b, 2c, 3a, and other CYP and XME genes regulated by the nuclear receptors constitutive androstane receptor (CAR) and pregnane X receptor (PXR) were differentially expressed following triazole exposure. Based on these results, we expanded on our original hypothesis that triazole hepatotoxicity was mediated by CYP induction, to include additional XME genes, many of which are modulated by CAR and PXR.

RMS comment: In mice exposed to 150 mg/kg bw/d myclobutanil, the majority of differentially expressed CYP genes were members of the CYP2 family: **Cyp 2c40** (↓); Cyp**2c55** (↑); Cyp **2d**26(↓); **Cyp3a11** (↑).
Myclobutanil altered additional phase I and II genes involved in metabolism and clearance of endogenous and exogenous compounds (XME genes) including aledhyde dehydrogenase5a1 (↓), glutathione S transferase (↑), and Ces2 (↑) which is critical for fatty acid and xenobiotic metabolism and transporter genes

Sic01a4 (↑), and Sic22a3 (↑). Regulation of many of these genes is mediated by the nuclear receptors CAR and PXR.

Exposure to myclobutanil induced expression of Constitutive Androstane Receptor (CAR) and pregnane X receptor (PXR) stimulated pathways which are involved in xenobiotic metabolism and clearance in the liver.

These results show that myclobutanil modulated mouse PXR receptor affecting the expression of metabolic genes. The increased expression of Cyp2c55 and Cyp3a11 is consistent with both PXR and CAR agonism. However, Cyp2c40, and XME were down regulated indicating possible CAR or PXR antagonism.

	0	**10**	**75**	**150 mg/kg bw/d**
Body weight (g)	33.86	34.28	33.45	34.77
Liver weight relative			↑13%	↑11%
centrilobular to midzonal hepatocyte hypertrophy: mild			*	*
Liver microsomal AROD activities:				
BROD	303	388	737*	831*
EROD (CYP 1A2, CYP2A6)	113	122	164*	197*
MROD(CYP 1A2)	181	209	247*	260*
PROD(CYP 2B1)	48	53	93*	110*

- Metabolism of myclobutanil and triadimefon by human and rat cytochrome P450 enzymes and liver microsomes (Barton et al., Xenobiotica, 2006, 36, 793-806)

Metabolism of two triazole-containing antifungal azoles was studied using expressed human and rat cytochrome P450s (CYP) and liver microsomes. Substrate depletion methods were used due to the complex array of metabolites produced from myclobutanil and triadimefon. Myclobutanil was metabolized more rapidly than triadimefon, which is consistent with metabolism of the n-butyl side-chain in the former and the t-butyl group in the latter compound. Human and rat CYP2C and CYP3A enzymes were the most active. Metabolism was similar in microsomes prepared from livers of control and low-dose rats. High-dose (115 mg kg-1 day-1 of triadimefon or 150 mg kg-1 day-1 of myclobutanil) rats showed increased liver weight, induction of total CYP, and increased metabolism of the two triazoles, though the apparent Km appeared unchanged relative to the control. These data identify CYP enzymes important for the metabolization of these two triazoles. Estimated hepatic clearances suggest that CYP induction may have limited impact in vivo.

In vitro half-lives of myclobutanil with expressed CYP isoforms and pooled microsomes

CYPs	Myclobutanil T1/2 minutes
Rat CYP 2B1	No metabolism was observed
Rat CYP 2C6	4.8
Rat CYP 2C11	4.4
Rat CYP 3A1	5
Rat CYP 3A2	10.4
Human CYP2B6	No metabolism was observed
Human CYP2C18	4.5
Human CYP2C19	3.9
Human CYP3A4	5.8
Human CYP3A5	65
Male rat liver microsomes	7
Female rat liver microsomes	12
Male human liver microsomes	40
Female human liver microsomes	27

The results in the table show that myclobutanil is metabolized by 2C and 3A subfamilies of CYPs. Rat CYP2B1 displays little if any ability to metabolize this triazole. Myclobutanil is metabolized by the human CYP3A4, which is the major human hepatic isoenzyme, however, another adult isoform CYP3A5 is also involved. The 2B sub-family appears not to mediate metabolism. Using commercially prepared pooled

microsomes, metabolism of myclobutanil was more important with rat microsomes than human and was similar in male and female humans and female rats but faster in male rats.

dose	Relative liver weight (%)	Total CYP (nmol/mg)
control	3.3	0.419
10 mg myclobutanil	3.3	0.489
150 mg/kg	4.0	0.699*

RMS comments: This study demonstrated that myclobutanil was predominantly metabolized by members of 2C and 3A subfamilies. After *in vivo* treatment, significant increase in total CYP content was evident after repeated high dose treatment of adult male rats. This induction was also reflected in significant increase in liver weight.

Biochemical and molecular studies were performed to identify that myclobutanil induces expression of genes that encode mammalian liver enzymes involved in xenobiotic elimination. Myclobutanil induces the levels of enzymes involved in oxidative metabolism (cytochromes P450 3), glutathione S transferase and small molecule transport (SiCo). These observations of multigene induction suggest the participation of a receptor-mediated pathway.
Myclobutanil differentially expressed 505 genes, increasing 136 and decreasing expression of 369 genes in adult liver mouse:
CYP genes: CYP 2c40 was suppressed, and CYP2c55 had increased expression suggesting a high important gene in metabolism of myclobutanil.

Five genes or cDNAs were expressed differentially: phosphomutase 2 was decreased, carboxylesterase2 (Ces2) was increased, 2 RIKEN cDNAs with similarity to oxidoreductases were increased and a RIKEN cDNA of unknown function was decreased.
Increased hepatic CYP450 activity levels resulted in increased AROD metabolism. Myclobutanil induced BROD, EROD, MROD and PROD metabolism without appearance of liver tumor after long term exposure.

In the testis, myclobutanil differentially expressed 623 genes, increasing 184 and decreasing the expression of 439 genes. Expression of CYP24 and CYP2b9 were induced.
CYPs have important function in both liver and testis upon exposure to myclobutanil.
CYP 2c40 is involved in inactivating exogenous substrates and in arachidonic acid into epoxyeicosatrienoic acid (potent vasodilatators) metabolism. This enzyme is expressed essentially in liver, kidney and intestine and brain of mice.
In testis: aromatase is the rate limiting enzyme responsible for the conversion of androgens to estrogens which are required for normal spermatid maturation.

- Comparison of lanosterol 14-α-demethylase (CYP51) of human and *Candida albicans* for inhibition by different antifungal azoles (Trosken et al., Toxicology, 228, 2006, 24-32)

Inhibition of fungal lanosterol-14α-demethylase (CYP51) is the working principle of the antifungal activity of azoles used in agriculture and medicine. Inhibition of human CYP51 may result in endocrine disruption since follicular fluid-meiosis activating steroid (FF-MAS), the direct product of lanosterol demethylation, is involved in the control of meiosis. To investigate the specificity of antifungal agents for the fungal enzyme, assays to determine inhibitory potencies of 13 agricultural fungicides and 6 antimycotic drugs were established. FF-MAS product formation was measured by LC–MS/MS analysis in the incubations using lanosterol as substrate. Recombinant human enzyme (hCYP51) was available from BD Gentest. CYP51 of *Candida albicans* (cCYP51) was co-expressed with *Candida tropicalis* oxidoreductase in the baculovirus system. IC_{50} values of 13 fungicides for cCYP51 ranged about six-fold (0.059–0.35 μM); for hCYP51 the range was about 30-fold (1.3–37.2 μM). The most favourable IC_{50} ratio human to *Candida* was observed for imazalil (440-fold), while the specificity of epoxiconazole and tebuconazole for cCYP51 was only by a factor of 10. For the antimycotic drugs, the range of IC_{50} values for cCYP51 was similar to those of fungicides (0.039–0.30 μM). For the inhibition of hCYP51, IC_{50} values split into two classes: the newer drugs fluconazole and itraconazole showed little inhibition (≥30 μM) while the older drugs were even more potent than the agricultural fungicides, with miconazole being the most potent (0.057 μM). No correlation was seen between the IC_{50} values determined for the two enzymes, indicating that a housekeeping gene can show significant diversity if inhibition is concerned.

Our data indicate that fungicide residues in food are unlikely to exert a relevant inhibition of CYP51 in humans whereas systemic use of some antimycotic drugs, e.g. ketoconazole or miconazole, should be carefully considered regarding disturbance of human steroid biosynthesis.

Comment from RMS: In this table, only 3 compounds are reported for comparison. The most potent inhibitor against human CYP51of the 3 was epoxiconazole suggesting a higher selectivity for myclobutanil for fungal CYP51 as for human as compared to propiconazole.

Inhibitory potency of azoles on human lanosterol-14α-demethylase (hCYP51) and CYP51 of *Candida albicans* (cCYP51)

Azole	IC50 human CYP51	IC50 *Candica* CYP51	ratio
myclobutanil	29 μM	0.14 μM	207
Epoxiconazole	1.95	0.22	9
propiconazole	8.25	0.15	55

- Disruption of testosterone homeostasis as a mode of action for the reproductive toxicity of triazole fungicides in the male rat (Goetz et al, 2007, Toxicol Sci., 2007 Jan; 95(1):227-39).

Triazole fungicides associated with a range of reported male reproductive effects in experimental animals were selected to assess potential toxic modes of action. **Wistar Han rats were fed myclobutanil (M: 100, 500, or 2000 ppm**), propiconazole (P: 100, 500, or 2500 ppm), or triadimefon (T: 100, 500, or 1800 ppm) from gestation day 6 to postnatal day (PND) 120. One male per litter was necropsied on PND1, 22, 50, or 92. Measurements included anogenital distance (AGD) at PND0, body and organ weights, serum hormone levels, age at preputial separation (PPS), sperm morphology and motility, and fertility and fecundity.
AGD was increased by the high dose of all three triazoles, indicating hypervirilization. Triadimefon delayed PPS, consistent with delayed puberty, at 1800 ppm.
Relative liver weights were increased at PND1, 50, and 92 by all three triazoles. Hepatocellular hypertrophy was present at PND50 from propiconazole and triadimefon and at PND92 from all three high-dose triazole treatments. Relative pituitary weights were decreased at PND92 by middle- and high-dose myclobutanil treatment. Absolute testis weights were increased at PND1 by myclobutanil, at PND22 by myclobutanil and triadimefon, and at PND50 by propiconazole and triadimefon treatment. Relative ventral prostate weights were increased at PND92 by myclobutanil and triadimefon treatment.
Serum testosterone was increased at PND50 by triadimefon and at PND92/99 by all three triazole treatments. Insemination and fertility were impaired by myclobutanil and triadimefon treatment. In addition to the reproductive system effects, total serum thyroxine levels were decreased at PND92 by high-dose triadimefon. These reproductive effects are consistent with the disruption of testosterone homeostasis as a key event in the mode of action for triazole-induced reproductive toxicity.
See below for more detailed report.

Overall conclusion from RMS:

Conazoles are a class of azole based fungicides having a common mode of antifungal action through inhibition of ergosterol biosynthesis. Some members of this class have been shown to be hepatotoxic and will induce mouse hepatocellular tumors and/or rat thyroid follicular cell tumors.
Many important advances have been made in the mechanisms that regulate the expression of drug metabolism enzymes and different receptors involved in these mechanisms and conazoles were studied in this domein.
CAR and PXR receptors activate the promoters of CYP2B and CYP3A gene expression by xenobiotics such as Phenobarbital-like compounds (CAR) and dexamethazone and rifampin-type of agents (PXR). The PPAR receptor is transcriptional activated by the promoters of CYP4A genes. CYP7A was recognized as the first target gene of liver X receptor (LXR) in which the elimination of cholesterol depends on CYP7A. Phenobarbital is a transcriptional inducer of the rat CYP2B1, CYP2B2 and CYP3A1 genes. CYP2C7 is also reported to be induced by PB as well as several phase II enzymes. PB is known to induce the expression of CYP2B gene by the CAR dependent mechanism.

Myclobutanil is not tumorigenic but induce hepatomegaly, increased cell proliferation, increased PROD, MROD and EROD activities, induction of cell proliferation and inhibition of cell apoptosis and decreased serum cholesterol and HDL.

After myclobutanil treatment, increased mouse liver expression of Cyp2b, Cyp2c and Cyp3a was reported. Induction of PROD was the most efficient. In the mouse Cyp1a1 and Cyp1a2 enzymes are generally associated with EROD and MROD activities while Cyp2b are associated with PROD activities and are well known to play important role in xenobiotic metabolism.
The Cyp2b and Cyp3a genes are regulated by constitutive androstane receptor (CAR) and pregnane X receptors (PXR) and have been widely studied for their high-level inducibility by Phenobarbital.
CAR activation and subsequent Cyp2b over expression have been linked to hepatic hypertrophy. The increased mouse liver and PROD activities after myclobutanil exposure coupled with the increased Cyp2b gene expression at the different time points are consistent with a CAR mediated hepatic hypertrophy.
Activation of PXR by myclobutanil leads to induction of rodent hepatic Cyp3a11. PXR activation *in vivo* regulates key steps involved in hepatic uptake, metabolism and biosynthesis of steroids and bile acids. It seems that PXR activation is required for xenobiotic induced hepatomegaly (Staudinger et al., Coordinate regulation of xenobiotic and bile acid homeostasis by PXR. Drug Metab Disp, 2001, 1467-1472). PXR is also activated by Phenobarbital. (Chang and Waxman, Drug Metab Rev., 38, 51-73, 2006).

RMS proposes that liver enlargement after exposure to myclobutanil is considered to be a xenobiotic induced adaptative effect. Myclobutanil stimulates enzyme induction and hepatic growth response is an adaptation to increased workload. Relative liver weight increases were found to be associated with histological evidence of hepatocyte hypertrophy. Necrosis was not reported and there was no transformation to tumour cells and there were no toxic responses.

B.6.6 Reproductive toxicity (Annex IIA 5.6)

B.6.6.2.1 Teratogenicity test by the oral route in the rat

Rat study, gavage, 31.3, 93.8, 312.6, 468.9 mg/kg bw/d (Costlow and Kane, 1984a)

In the DAR it was reported that:

Fetal morphological observations: table B.6.6.2.1-2.
The incidence of the 7th cervical rib and 14th rudimentary ribs was significantly increased at 312 and 468.9 mg/kg bw/d. These data suggested that myclobutanil was fetotoxic at maternal toxic doses of 312 and 468 mg/kg bw/d.
a NOAEL maternal tox= 94 mg/kg bw/d was proposed based on clinical signs of toxicity occurring at 312.6mg/kg bw/d.
NOAEL developmental tox = 31.3 mg/kg bw/d taking into account the altered viability index at 93.8 mg/kg bw/d (needs a classification as Repro Cat 3, R63). Increased incidences of 14th rudimentary and 7th cervical ribs were observed at maternal toxic doses of 312 and 469 mg/kg bw/d.

Table B.6.6.2.1-2: Incidence of developmental effects in litters from mothers exposed to myclobutanil

Target organ/dose mg/kg bw/d		Hist.cont	**31.3**	**93.8**	**312.6**	**468.9**
Skeletal variations:	N° affected fetuses/n° affected litters					
N° litters examined	22		24	21	23	22
7th cervical ribs	3/2	1/1	0/0	3/3	17/10*	45/14*
14th rudimentary ribs	1/1	61/23	4/3	1/1	17/8*	72/18*
Any rib variation	8/5	443/176	7/6	11/7	34/16*	72/20*
Any reduced ossification	150/22		103/24	93/18	123/18	125/22
Soft tissue malformation:						
hydrocephaly						2/2
craniorachischis						1/1
Skeletal malformation						
Atlo-occipital anomaly			1/1			1/1
Vertebral centra bipartite						1/1
Total external malformations	0/0		1/1	2/2		1/1

Target organ/dose mg/kg bw/d		Hist.cont	31.3	93.8	312.6	468.9
Total skeletal malformations	0/0		1/1	0		2/1
Total soft tissues malformations	0/0			2/2		2/2
Total malformations	0/0		2/2	3/2		4/4

*Significantly different from controls

The company concluded that myclobutanil is not toxic for development in rats under these experimental conditions.
For the RMS, the classification of increased incidence of a 7th cervical rib as variation is an area of uncertainty. There is an issue in terms of length of the additional structure. If a cervical rib remains very short, it might be less harmful than if it is long, or later becomes long. length should have been recorded. In human, cervical ribs cause problems with brachial plexus and subclaviar blood vessels. This should be discussed at ECB (Ispra).

In december 2005, the company provided a re-evaluation of the skeletal speicmens using length criteria. Only ribs whose length was more than twice their width were considered supernumerary ribs, while shorter ribs were considered normal.

- **Re-analysis of selected skeletal findings from a teratology study with RH-53,866 (myclobutanil) in rats (Carney et al., 2005)**

Findings:

In the 31.3, 93.8 and 312.6 mg/kg bw/d groups, re-evaluation using the length criteria reported in material and methods, revealed no cases of cervical rib or 14th rudimentary rib, whereas one control fetus had a 14th rudimentary rib which met the size criteria. In fact, many of the original supernumerary rib observations were based on small, pinpoint sites of ossification. In contrast to the original report, which indicated an effect on these two alterations at dose level of 312 mg/day, the re-evaluation based on the length criteria clealry indicated an absence of true supernumerary ribs at this dose level.
Re-evaluation of the high dose group skeletal specimens revealed 6 fetuses in 6 litters with 14th rudimentary rib, resulting in an incidence of 27% of litters and 3% of fetuses, which was much lower than originally reported (81% of litters and 35% of fetuses). Nonetheless, the incidence of the 14th rudimentary rib based on length cirteria remained significantly elevated relative to the control group (4.5% of litters, 0.4% of fetuses). There was also one additional high-dose fetus with a full 14th rib.
True 7th cervical ribs were found in 4 high-dose group fetuses from 3 litters, resulting in an incidence of 13% of litters and 2% of fetuses, which was much lower than originally reported (63% of litters, 22% of fetuses). The difference relative to controls was not statistically significant.
The increases in 7th cervical and 14th rudimentary rib in the high dose-group were considered to be treatment-related.

Table B.6.6.2.1-2: Incidence of 7th cervical rib and 14th rudimentary rib as evaluated according to the length criteria.

Target organ/dose mg/kg bw/d	0	31.3	93.8	312.6	468.9
Skeletal variations:	N° affected fetuses/n° affected litters				
N° fetuses / litters examined	223/22	213/24	185/21	200/23	201/22
7th cervical ribs	0/0	0/0	0/0	0/0	4/3
14th rudimentary ribs	1/1	0/0	0/0	0/0	6/6*

*statistically different from controls

Conclusion: according ot the company, the presence of maternal toxicity was seen during the critical period for supernumerary rib induction, and these skeletal alterations were considered to represent fetotoxicity , but not teratogenicity, associated with maternal toxicity.

Comment from RMS: as maternal toxicity was apparent at top dose, it can be considered that these effects are secondary to maternal toxicity.

Material and methods: all fetal skeletal specimens from the original study were retrieved from long-term storage, and those specimens with an obsrevation of 7th cervical rib and/or 14th rudimentary rib were re-evaluated based on lentgh . As per standard procedure in our laboratory,

supernumerary rib with a length which was less than twice its width was considered normal, whereas larger ribs, defined as having a length equal to or more than twice their width, remained classified as 7th cervical or 14th rudimentary ribs. These data from this re-evaluation were then statistically analyzed using the Censored Wilcoxon test, with the litter serving as the unit of analysis.

Conclusion of the RMS:

In summary, re-evaluation of the skeletal specimens using length criteria similar to that recommended in several recent publications showed a complete lack of true 7th cervical or 14th rudimentary ribs at dose levels of 31.3, 93.8 and 312.6 mg/kg/day. However, there remained a slight, but statistically identified increase in true 14th rudimentary ribs at the highest dose level (468.9 mg/kg/day), along with a marginal increase in 7th cervical rib which was not statistically identified. The incidences of both skeletal alterations were just slightly above expected control incidences based on published data using similar rib length criteria and, therefore, were considered to be treatment-related effects. Given the marginal nature of these supernumerary rib increases, the lack of any corresponding pattern of fetal malformation, and the presence of maternal toxicity during the critical period for supernumerary rib induction, these two skeletal alterations were considered to represent fetotoxicity, but not teratogenicity, associated with maternal toxicity.

Data from the open literature:

- Disruption of Testosterone Homeostasis as a Mode of Action for the Reproductive Toxicity of Triazole Fungicides in the Male Rat (Amber K. Goetz, Hongzu Ren, Judith E. Schmid, Chad R. Blystone , Inthirany Thillainadarajah, Deborah S. Best, Harriette P. Nichols, Lillian F. Strader, Douglas C. Wolf, Michael G. Narotsky, John C. Rockett and David J. Dix. Toxicological Sciences 2007 95(1):227-239)

Findings:

According to the authors: Dams exposed to myclobutanil at 2000 ppm had reduced food consumption during week1-2 of lactation.
Significant body weight loss was observed at 2000 ppm.
Litters exposed to 2000 ppm had decreased survival rates with deaths. Anogenital distance was increased at 2000 ppm. Relative liver weight was increased at PND1 at top dose. Absolute testis weight was increased after 100 and 2000 ppm at PND 1 and at PND 22 following 500 ppm. Relative and absolute ventral prostate weight was increased following 500 ppm. Pituitary weight was decreased at PND 92 after 500 and 2000 ppm. At PND 92, at 2000 ppm, mild centrilobular hepatocyte hypertrophy was observed. Serum testosterone was increased at PND 92 after 2000 ppm. Serum levels of estradiol and LH were unaffected. Total T4 was not affected. There were no significant differences in sperm head or tail morphology. Insemination index was reduced at top dose. Fertility index was reduced in mating pairs of untreated females at 500 and 2000 ppm. There were no significant effects on total number of implantation sites, live or dead fetuses/embryos or number of resorptions.
Insemination and fertility were impaired by myclobutanil treatment. These reproductive effects are consistent with the disruption of testosterone homeostasis as a key event in the mode of action for triazole-induced reproductive toxicity.

Table B.6.6.2.1-3: experimental results after exposure of dams to myclobutanil

	0	**100**	**500**	**2000 ppm**
Compound intake: mg/kg bw/d				
GD6-PND0	0	8-8.1	38.8	141.3-149.9
PND 1-22	0	8.1-19.1	39.4-93.8	155-347
Food consumption:				
GD6-PND0				(↓4%)
PND1-22				(↓4%)
PND 30-36				↓23%
PND 44-50				↓10%
PND58-64				↓15%
PND 65-71				↓11%
Body weight:				
PND22 (lactation)				↓4%

PND 23-29				↓6%
PND 30-36				↓8%
PND37-92				↓12%
Litter size	9.8	10.1	9	7.5
Total litters	27	17	19	30
Total litters with deaths	3	2	4	18
Litters lost entirely	1	1	1	1
Total offspring	276	172	171	230
% survival	95.6	92.4	93.5	82.6*
Liver weight: rel				
PND 1				↑13%
PND 22				No effect
PND 50				↑11%
PND 92				↑9%
Testis weight rel				
PND 22				↑17%
PND 50				↑20%
Pituitary weight :				
PND92			↓18%	↓11%
Serum testosterone				↑
N° inseminated females (/n° mated)%	90.5	100	63.6	31.3*
N° pregnant females	19/19	11/11	6/11*	4/16*
Fertility index	100	100	54.5*	25*
Post implantation loss: %	6.61	11.76	8.41	9.42
Sperm morphology % normal	88	88	87	87
Sperm motility	136.3			134
Anogenital distance				↑

↓ statistically significantly decreased or increased; () not statistically modified

RMS evaluation: myclobutanil produced toxic effects at 2000 ppm as suggested by the decreased food consumption, and reduced body weight at top dose of 2000 ppm. Only 1 male/litter was necropsied on PND 1, 22, 50 or 92. From the reported results it is not always possible to conclude on which animals the reported analyses were performed. So, it appears that systemic toxicity is evident at 2000 ppm but it cannot be concluded from this study if the reproductive toxicity is a primary effect or an effect related to systemic toxicity.
RMS considers that this study does not provide suitable additional information.

Material and methods:
Dams were allowed to deliver naturally for the F_1 offspring. On PND8, litters were weighed and then culled to eight pups per dam, retaining males preferentially, to maximize uniformity in growth rates. Survival rates of offspring were based on percentage of animals remaining past PND8. The ratio of alive to dead male and female pups per treatment group was analyzed using Fisher's exact test, measures with $p \leq 0.05$ were considered significant. F_1 offspring were housed with their respective mothers until weaning at PND23. Males and females were then removed from the dams and housed by treatment in same-sex pairs until PND50. Males were single housed after PND50, females remained housed in pairs. Control animals were fed 5002 Certified Rodent Diet with acetone vehicle added. Treatment groups received feed containing either myclobutanil (100, 500, or 2000 ppm). Dams began treated feed diets on GD6, continuing through gestation, parturition, and lactation. The F_1 generation continued on the same treated feed diets upon weaning at PND23. F_1 offspring feed intake and body weights were measured weekly until necropsy. One male from each litter was taken to necropsy at PND1, 22, 50, or 92 to assess affects on select organ weights, histology, and hormone measures. On PND0, pup body weight and anogenital distance (AGD) were measured, and footpads were tattooed for identification. F_1 males were examined for preputial separation (PPS) beginning on PND38, continuing daily until complete cleavage of the epithelium lining the prepuce of the penis was observed indicating onset of puberty was achieved. Body weights were measured on the day of PPS. On PND1, 22, 50, or 92 whole-body weights were measured, and then brain, hypothalamus, hippocampus, pituitary, thyroid, liver, testis, epididymis, ventral prostate, and seminal vesicles were removed, weighed. Blood was collected at PND22, 50, 92, and 99. One testis and epididymis from each male at PND22, 50, and 92 necropsies were used for morphology analysis.Brain, pituitary, thyroid, liver, testis, epididymis, and ventral prostate were collected for histological evaluation from each necropsy time point. Blood samples were collected and set on ice.

Serum was prepared from the blood on the same day of necropsy and. Estradiol, testosterone, total triiodothyronine (T_3), and total thyroxine (T_4) levels were assayed in duplicate.Sperm was preparated for morphology and motility. Insemination and Fertility Indices were controlled. Ejaculated sperm countswas performed after natural breeding.

B.6.5 long term toxicity and carcinogenicity(Annex IIA 5.5)

RMS suggests to the company to clarify the relevance to humans of the mechanism inducing specific testicular atrophy observed in rats to conclude that no classification is required.

The company provided the following:

Testicular atrophy (and associated sequel) were observed only in the male rat at systemically toxic doses, but not in any other species studied (mouse 2-year carcinogenicity study and dog 1-year toxicity study) at comparable doses. In addition, this finding was present only in the 2-year carcinogenicity study and the second generation of the 2-generation reproduction toxicity study, but not in any shorter term rat studies. It should be noted that testicular atrophy is a common finding in the ageing rat.

In the 2-year carcinogenicity study, the incidence of bilateral testicular atrophy was increased at 39.2 mg/kg bw/day and the effect appeared to be progressive with time and dose (Table 1). This was first noted at the 12-month time-point. The incidence of unilateral testicular atrophy was comparable to controls at each time-point. The gross pathology findings of reduced testis size did not directly correlate with the histopathological findings. Testes weights were decreased (12-25% at the top dose) with increasing time. Microscopically, the seminiferous tubules were frequently devoid of spermatid formation and germinal epithelial cells. In severe cases, only Sertoli cells remained. These findings account for the gross appearance of atrophy. The testicular effects in the control and low dose (2.5 mg/kg bw/day) were comparable, and no abnormalities were seen at 3 and 6 month time-points at any dose level. The incidences of other findings in the testes, such as polyarteritis, did not show the same pattern of dose or time relationship. It should be noted that atrophy was not observed histopathologically at 106 mg/kg bw/day in the MTD 2-year rat carcinogenicity study, though aspermatogenesis and hypospermia were seen.

Table 1. Histopathological alterations in the rat 2-year carcinogenicity study.

Time-point	**12 Months**				**Study 2[a]**	**17 Months**				**24 Months**				**Study 2[a]**	**Died/Sac Moribund**				**Study 2[a]**
Dosage (mg/kg bw/day)	0	2.5	9.8	39.2	106	0	2.5	9.8	39.2	0	2.5	9.8	39.2	106	0	2.5	9.8	39.2	106
Testes (No. of tissues examined)	**20**	**19**	**20**	**20**	**10**	**18**	**18**	**18**	**18**	**17**	**19**	**20**	**22**	**16**	**35**	**35**	**32**	**30**	**34**
Testes weight (g)	3.751	3.662	3.524	**3.300***		3.431	3.393	3.655	3.017	3.223	3.006	**2.491***	**2.430***						
% decrease			6	**12**				+6.5	**12**		6.7	**22.7**	**24.6**	23					
Testes:body weight ratio	0.556	0.512	0.516	0.507		0.434	0.449	0.470	0.389	0.492	0.488	0.444	0.388						
Gross pathology:																			
Reduced size	-	1	1	3		4	3	1	7	-	2	7	6		2	6	2	7	
	-				5/5					-				4/5	5/5				13/15
Soft testis	-				4/5					-				0/1	5/5				13/13
Histopathology:																			
Polyarteritis	-	-	-	1						3	1	4	5		1	1	5	4	
Polyarteritis – bilateral															0	0	2	0	
Periarteritis						1	-	-	1										
Atrophy – unilateral	-	1	-	-		2	2	-	1	2	3	6	2		6	4	5	5	
Atrophy – bilateral	**-**	**-**	1	**3**		2	2	**-**	**4**	2	1	**5**	**12**		1	4	**10***	**12***	
% incidence			5	**15**		11	11		**22**	12	5	**25**	**55**		3	11	**31**	**40**	
Orchitis															1	0	0	0	

Time-point	12 Months				Study 2[a]	17 Months				24 Months				Study 2[a]	Died/Sac Moribund				Study 2[a]
Dosage (mg/kg bw/day)	0	2.5	9.8	39.2	106	0	2.5	9.8	39.2	0	2.5	9.8	39.2	106	0	2.5	9.8	39.2	106
Arterial mineralisation										-	-	1	-		0	1	0	2	
Oligospermatogenesis															1	0	0	2	
Bilateral aspermatogenesis	-				6					(0/17)				4	(2/33)				12
Unilateral hypospermia	-				2					(1/17)				2	(3/33)				4
Tubular necrosis															0	0	0	1	
Bilateral seminiferous tubule atrophy						-	-	-	2										
Scrotal varicocele	-	-	-	1															

[a]Study 2 is the MTD 2-year rat carcinogenicity study, at 0 and 106 mg/kg bw/day in males

In the 2-generation reproductive toxicology study, similar testicular effects were observed in the second generation adult males, but not in the P1 generation males. The changes were primarily increased incidence of diffuse testicular atrophy, prostatic atrophy, necrotic spermatocytes/spermatids and decreased spermatozoa in the epididymides, as shown in Table 2 below:

Table 2: Incidence of histopathological changes in the P2 rats

Sex	**Males**			
Exposure Conc. (ppm)	**0**	**4**	**16**	**80**
Number of Animals Examined	25	25	25	25
Gross pathology: small flaccid testes	0	1	1	**8**
Testes (No. of tissues examined)	25	25	25	25
Multifocal Atrophy – unilateral	0	2	1	2
Multifocal Atrophy – bilateral	3	2	3	3
Diffuse Atrophy – unilateral	0	0	1	**4**
Diffuse Atrophy – bilateral	0	1	0	**4**
Diffuse necrosis - unilateral	0	1	0	0
No. of rats with testicular lesions	3	5	5	**11**
Epididymides (No. of tissues examined)	25	25	25	25
Necrotic spermatocytes/spermatids – unilateral	0	0	0	**5**
Necrotic spermatocytes/spermatids – bilateral	2	3	2	**8**
Decreased spermatozoa – unilateral	0	0	1	1
Decreased spermatozoa – bilateral	1	2	0	**8**
No. of rats with epididymal lesions	2	3	3	**13**
Prostate (No. of tissues examined)	25	25	25	25
Atrophy	2	1	0	**11**
Chronic interstitial prostatitis	4	2	2	0
Focal suppurative prostatitis	0	1	0	0
Focal hyperplasia	0	0	1	0

This pattern correlates with the more pronounced evidence of systemic toxicity in P2 animals relative to the P1 animals. For example, histologic changes in the liver were seen in the middle-dose P2 males, but not in P1 males at 16 mg/kg bw/day. Reduced weight gain was also seen in P2, but not P1, 80 mg/kg bw/day males.

Impact on fertility. A total of four matings (two litters per generation) were performed in the study, thus providing ample data to assess fertility. Consistent with the lack of histopathological changes in the male reproductive organs, there was no convincing evidence of an effect on fertility in the F1 generation. Although the number of F1a high-dose females giving birth (20) was slightly lower than control (23), this was not repeated in the F1b litter. In fact, the number of high-dose females giving birth following the F1b mating (23) was slightly higher than that of controls (22). Regarding male fertility, individual animal data in the study report were used to calculate the number of males which successfully sired a litter of viable pups. It was found that 25/25 (100%) of the high-dose group P1 males were fertile vs. 24/25 (96%) in the control P1 males. These data clearly indicate that there were no adverse effects on fertility among the P1 males and females.

Table 3: Myclobutanil – Summary of fertility data for P1 animals

Indices	F1a				F1b			
	Dosage (mg/kg bw/day)				Dosage (mg/kg bw/day)			
	0	4	16	80	0	4	16	80
Number of males	25	25	25	25	25	25	25	25
Number of females	25	25	25	25	25	25	25	25
Females mating	25	25	25	24	25	23	25	22
Females giving birth	23	24	22	**20**	22	22	23	23
Females weaning litters	23	24	22	19	22	22	22	23
Days to mating	3.1	2.8	2.6	3.3	2.4	2.6	2.7	2.1
Gestation period (days)	21.8	21.8	21.8	22.1	21.9	21.8	22	21.9

Stats: No statistically significant findings

The two matings of the P2 adult animals revealed a decrease in the number of high-dose group females giving birth relative to controls (Table 4). Again, male fertility indices were not provided in the study report, but were calculated based on individual animal data shown in the report appendices. The percentage of high-dose P2 males which successfully sired a litter (18/25, 72%) was decreased relative to controls (24/25, 96%). Interestingly, there was a very close individual animal correlation between histopathological changes in the testes and epididymides, and the failure of males to sire a litter. Six of the seven high-dose males that failed to sire a litter exhibited these histopathological changes at necropsy. This might suggest that the failure to sire a litter was secondary to the testicular atrophy and associated histopathological changes.

Table 4: Myclobutanil - Summary of fertility data for P2 animals

Indices	F2a				F2b			
	Dosage (mg/kg bw/day)				Dosage (mg/kg bw/day)			
	0	4	16	80	0	4	16	80
Number of males	25	25	25	25	25	25	25	25
Number of females	25	24	25	25	25	24	25	25
Females mating	25	23	23	22	24	21	25	21
Females giving birth	23	23	24	**20**	23	22	25	**17**
Females weaning litters	22	23	23	18	22	21	24	15
Days to mating	2.2	3.1	2.8	3.0	3.0	3.4	2.9	4.4
Gestation period (days)	21.7	21.7	21.9	22.2	21.7	22.0	21.8	21.7

Stats: No statistically significant findings

Litter data. At 80 mg/kg bw/day, the number of pups born dead was increased in all four matings. However, this appeared to be a marginal effect, as the percentage of pups born alive was no lower than 94.7%, vs. a low of 98.6% among the controls. The incidence of dead pups was not markedly different between the first and second generations (Table 5 and 6). The total number of pups per litter (i.e., includes live and dead pups) was not affected by treatment in either the F1a or F1b matings. In the F2a and F2b matings, total number of pups per litter was statistically decreased at the high dose level of 80 mg/kg bw/day. However, the number of pups per litter in the high-dose F2b litter (13.4) was similar to the number in the F2a controls (13.8), again suggesting that this effect was marginal. There was no increase in pup mortality from postnatal day 4 onward, although pup body weights were decreased in the high dose group in all matings.

The weight of evidence suggests that the increased number of pups born dead, and the slight decrease in litter size (P2 only) are most likely the result of post-implantation loss and/or perinatal death, rather than a consequence of impaired fertility. Again, the decrease in the number of mated females which delivered in the

P2/F2a mating, was similarly seen for the P1/F1a mating (Table 3 and 4), and therefore cannot be directly attributed to the testicular effects in the P2 males. Furthermore, changes in fertility were not noted in a dominant lethal study in which a single gavage dose of 0, 10, 100 or 735 mg/kg bw myclobutanil did not result in a dominant lethal effect through 8 weeks of mating. Dominant lethal studies are designed to detect effects on pregnancy rates, live fetuses/litter, total implants and fetal deaths. There was no indication of a dosage-dependent increase in fetal death, even at an adult-lethal dosage. Also, a rat developmental toxicity study with myclobutanil found decreased embryo viability at oral gavage doses of 93.8 mg/kg bw/day or higher. Although the dose levels cannot be directly compared due to the difference in dose-rate in the two studies (i.e., bolus effect in gavage studies vs. slower rate of intake in the diet study), the effects are qualitatively consistent.

Table 5: Myclobutanil – Summary of litter data for P1 animals

Litter data	**F1a**				**F1b**			
	Dosage (mg/kg bw/day)				**Dosage (mg/kg bw/day)**			
	0	**4**	**16**	**80**	**0**	**4**	**16**	**80**
Total pups/litter at birth	13.7	12.8	13.7	12.3	13.0	13.3	13.7	14.2
Sex ratio (M/M+F) at birth	0.44	0.47	0.50	0.53*	0.45	0.46	0.45	0.52
No. pups born dead	3	4	9	12*	0	6	9*	16*
Percent born alive	99.1	98.7	97.0	95.1	100	97.9	97.1	95.1
Litter size - live pups								
Birth	313	302	293	233	287	292	315	327
Day 4 pre-cull	311	297	291	227	258	249	243	282
Day 4 post-cull	226	224	209	177	208	200	202	211
Day 7	226	224	209	177	206	198	200	211
Day 14	225	224	209	177	205	198	199	210
Day 21 (weaning)	225	224	209	177	204	198	196	210
Body weight (g)/pup								
Day 0	6.0	6.1	6.2	6.3	5.9	6.0	6.1	5.9
Day 4 pre-cull	9.6	9.9	9.6	9.4	9.5	9.0	9.4	8.5
Day 7	15.3	15.1	15.0	14.3**	15.2	14.3	14.9	13.1 **
Day 14	29.6	29.6	29.2	26.7**	30.4	29.1	29.8	26.9 **
Day 21 (weaning)	45.7	45.9	44.4	41.9**	46.6	45.6	46.2	42.2 **

*Stats: *p<0.05 for combined sex; **p<0.05 for each sex*

Table 6: Myclobutanil - Summary of litter data for P2 animals

Litter data	F2a				F2b			
	Dosage (mg/kg bw/day)				Dosage (mg/kg bw/day)			
	0	**4**	**16**	**80**	**0**	**4**	**16**	**80**
No. pups/litter at birth	13.8	13.8	13.1	**11.4***	15.4	14.8	13.8	**13.4***
Sex ratio (M/M+F) at birth	0.53	0.51	0.45	0.46	0.49	0.51	0.51	0.46
No. pups born dead	6	3	1	**13***	5	6	3	**12**
Percent born alive	98.7	99.1	99.7	94.7	98.6	98.2	99.1	95.6
Litter size - live pups								
Birth	314	314	314	216	349	319	341	218
Day 4 pre-cull	276	269	273	193	343	306	339	207*
Day 4 post-cull	209	215	216	169	230	207	240	155
Day 7	209	213	216	169	230	202	240	154
Day 14	208	213	216	169	230	202	240	154
Day 21 (weaning)	208	213	216	169	220	202	239	144
Body weight (g)								
Day 0	5.8	6.1	6.2	6.2	6.0	5.9	6.1	5.8
Day 4 pre-cull	9.2	9.6	9.9	9.2	9.1	9.5	9.1	8.7
Day 7	14.9	15.1	15.2	13.4	14.5	15.0	14.7	13.3**
Day 14	29.1	29.2	29.2	25.3**	29.4	30.2	28.7	26.2**
Day 21 (weaning)	45.3	45.5	44.6	40.2**	46.5	48.1	46.0	41.8**

*Stats: *p<0.05 for combined sex; **p<0.05 for each sex*

In summary, there is no clear evidence that the testicular atrophy observed only in aged rats (first noted at 12 months in the 2-year rat carcinogenicity study) and P2 males (following 27 weeks exposure in the 2-generation reproduction study) caused impaired fertility. Effects observed in the top dose group of the 2-generation study included reduction in the number of viable foetuses and numbers of females delivering, and an increased number of pups born dead. It is not clear if these effects are related to impaired fertility or to post-implantation effects. However, the rat (and rabbit) developmental toxicity study clearly demonstrated embryo/foetotoxicity with reduced viability index, and increased number of resorptions. If these dams in the developmental study had been allowed to deliver their litters, a similar pregnancy outcome may have occurred as that observed with the 2-

generation study. This information would suggest that the effects observed in the 2-generation study were due to developmental toxicity and not impaired fertility.

Therefore, the relevance to humans of this species-specific testicular atrophy remains unclear.

RMS can agree with the considerations of the company.
From Paper 19, it was concluded that the testes atrophy is observed at systemic toxic doses and does not require classification.

B.6.9.3 Observations on exposure of the general population and epidemiological studies(Annex IIA 5.9.3)

In the original DAR, no information was provided.

The company provided the following in November 2006:

Introduction:

Myclobutanil is a triazole fungicide which was manufactured previously by contract manufacturer Rhodia Chirex in the U.K., and since late 2002 by contract manufacturer Kemira Fine Chemicals in Finland. Myclobutanil is repackaged in Barranquilla, Columbia. Medical surveillance data on 8 employees have not shown any abnormalities to suggest adverse health effects; there have been no incidents or allegation of adverse effects in this operation. Myclobutanil was bottled briefly in San Lorenzo, Argentina in 2002. No medical surveillance has been conducted on the 7 workers involved. No medical surveillance data on these manufacturing personnel are available.

PLANT REPORT MOZZANICA/ MYCLOBUTANIL MANUFACTURING / FORMULATION

Report Date: 11-7-2006

This report covers medical surveillance data available from the manufacturing/formulation of myclobutanil at Mozzanica, Italy over the time span 2000-2005 and covers data for 25 workers. Dr. Leghissa confirmed that for all 25 workers there are no health effects related to working with myclobutanil. Description of the Mozzanica medical surveillance exams is below.

Physical exam done every year.
Blood samples done every two years
Urinalysis - every two years
Spirometry - for those working in the plant
Audiogram only employees in the plant
ECG - 40/50/60 (every 3,2,1 yrs)
Vision testing - for administrative employees, also by eye specialist.
Serum chemistry: same content as Dow**, but also hepatitis marker done - prevalence of Australia A.G. is high in the area.
No stress assessment.

**Dow Health Assessment components:
Elements of the HSS Exam
The following elements shall be included in the Health Surveillance and Screening Examination:

- The Health Assessment Program information sheet
- Periodic Health questionnaire
- Physical measurements
  - Height, weight, and body mass index*
  - Pulse and blood pressure*
  - Waist measurement*
- Laboratory tests
  - Urinalysis
  - Complete blood count

Serum chemistry: Cholesterol, HDL, LDL, triglycerides (lipid panel)*
(fasting) ALT, AST, GGT, and alkaline phosphatase (AP)
glucose* and creatinine

- Audiogram (testing determined from questionnaire)
- Forced expiratory spirogram including FVC, FEV1.0, & FEV1.0/FVC ratio (only for those working in a manufacturing or lab setting)
- Electrocardiogram (40, 50, and 60 years of age)*
- Vision (testing determined from questionnaire)*
- Fecal occult blood testing (FOBT) (i.e. Stool hemoccult card) for employees 50 years old and older (where it is available)
- Personal Stress Assessment (PMI) based on Regional implementation strategy
- Counseling, resource referral and follow up by a Health Professional (including information about health screening tests conducted by a personal physician) at locations with on-site Dow Health Services *
- Physical examination, if clinically indicated

*Health screening tests

B.6.10.3 Acute reference dose (ARfD)

In the original DAR, RMS proposal was as following:

In the developmental rat study, embryotoxicity occurred at a dose of 93.8 mg/kg bw/d with a decreased viability index and increased resorption rate. Maternal toxicity was evident at 312 mg/kg bw/d. the NOAEL developmental toxicity was 31.3 mg/kg bw/d.

The rat developmental toxicity study is the most appropriate to use for setting the ARfD. A NOAEL= 31.3 mg/kg bw/d was established in this study due to embryotoxic effects (altered viability index). The findings are considered the most sensitive short-term, treatment related effect with myclobutanil, with possible relevance to humans. Based on this NOAEL and an assessment factor of 100 for inter- and intra-species extrapolation, the proposed ArfD is:

ArfD= 0.31 mg/kg bw/d

There is a 300-fold margin between the proposed ArfD and the LOAEL for developmental effects in the rat developmental toxicity study.
Same proposal from the company.

This proposal was accepted at Praper 19.

Comments from the Reporting Table:

ARfD: 2(27) UK: The effects observed in the multigeneration study (including increased numbers of stillborn and decreased numbers of females delivering) are considered potentially relevant to acute exposure, and thus the UK considers that the ArfD should be derived using the NOAEL from this study.
With a proposed ARfD of 0.16 mg/kg bw, there is a margin of 200 on the NOAEL for developmental effects. This should give an adequate margin.

According to the company:

An acute reference dose is required for substances that may be considered to represent an acute hazard (e.g. acute oral LD_{50} < 1000 mg/kg bw; WHO, 2004). An ARfD is the amount of a substance that can be ingested in a period of **24 hours or less** without appreciable health risk to the consumer (SCP, 2002; WHO, 2004; Solecki *et al*., 2005). As such, it should be based on a relevant toxicity study representative of a single daily dose. A specific study designed to enable an accurate ARfD to be set for myclobutanil has not been conducted.

The EU guidance document (7199/VI/99 rev. 5, 2001) indicates that sub-acute studies may represent one of the most adequate sources of data for setting an ARfD (if they are OECD compliant) and that a teratology study falls into this category when it contains "developmental effects, except when these are clearly a consequence of maternal toxicity".

The utilisation of a sub-chronic study would be inappropriate considering the adequate availability of studies of acute and sub-acute durations which cover the principle effects observed with myclobutanil. The use of the 2-generation reproduction study is also considered inappropriate, as the duration of exposure far exceeds the representative period for exposure of "24 hours or less", and it is highly unlikely that a single exposure during the 2-generation study would lead to the additional adverse effects noted in this study (which are not already covered by the developmental study). The repeat dose LOAEL for the effects observed in the 2-generation study is 80 mg/kg bw/day, and thus the rat developmental toxicity repeat dose NOAEL (31.3 mg/kg bw/day) is >2-fold lower than this value and adequately protects against the LOAEL effects.

It is considered appropriate to use the NOAEL from the developmental toxicity rat study (based on 10 days daily exposure by gavage), as dosing occurs during a sensitive period, and would directly reflect on the adverse effects noted in these studies.

Therefore, the rat development study is the most appropriate to use for setting the ARfD on the basis of the embryotoxic effects, and thus this adequately covers the effects of concern from the 2-generation study.

In summary the ARfD is: 31.3 mg/kg bw/day / 100 (SF) = **0.31 mg/kg bw**.

References:

FAO/WHO, 2004. Pesticide Residues in Food – 2004. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues, Rome, Italy, 20 – 29 September 2004. FAO Plant Production and Protection Paper, 2004.

Guidance for the setting of an acute reference dose (ARfD). Draft Working Document 7199/VI/99 rev. 5 of 5 July 2001.

Scientific Committee on Plants SCP/Guide-ARFD/002-Final (2002). Opinion of the Scientific Committee on Plants on the Draft Guidance Document for the Setting of an Acute Reference Dose (ARFD). Adopted 18 July 2002.

Solecki *et al*. (2005). Guidance on setting of acute reference dose (ARfD) for pesticides. *Food Chem. Toxicol*., **43**; 1569-1593.

B.6.10.4 Acceptable operator exposure level (AOEL)

In the original DAR, RMS propose to set the AOEL = 0.16 mg/kg bw/d based on the relevant NOAEL for liver toxicity at 400 ppm (14.3 mg/kg bw/d) and on the 2-generation rat study NOAEL = 200 ppm (16mg/kg bw/d) where an increased incidence of still born fetuses and decreased number of females delivering litters were observed.

Comments from the Reporting Table:

2(24) DK disagrees with the proposed AOEL. We propose to base the AOEL on the NOAEL from the long-term rat study where effects are seen on the testes at 9.8 mg/kg/d already after 1 year. And as the effects are serious we propose to use a SF of 300. I.e. the AOEL will be 0.03 mg/kg bw/d.

2(28) UK: Due to the magnitude of the liver weight effects in females at 400 ppm in the 1 year dog study, combined with the increased SAP activity and histopathology, the UK considers that this study derives a NOAEL of 100 ppm. This is lower than that obtained in the rat multigeneration study, and should be used in the derivation of the AOEL.

According to the company:

The **European guidance requires** us to consider the application window which defines the maximum duration and frequency of exposure that any operator might receive. This is the basis upon which we can decide the appropriate toxicology study to use to set the AOEL. These 2 elements enable us to assess the risk which is a function of the exposure and hazard.
The application window is:

- Frequency and duration of use of a product
- Not time period stated on the label
- Country and region specific
- Specific to growth stage of application
- Determined by climatic conditions during a year

Therefore, collectively, these conditions restrict the application window to a very narrow time period which is probably not more than 4 weeks/year, certainly is less than 3 months/year and, also, **intermittent** during these time periods.

The Guidance for the Setting of Acceptable Operator Exposure Levels (AOELs) clearly states that the definition of the toxicity profile should be relevant for **frequency and duration of exposure of operators** (including bystanders and re-entry workers), associated with the handling and use of the plant protection product. Based on this principle, the NOAEL from the 1-year dog study is not appropriate for AOEL setting as there were **no adverse findings in the first 3 months** of this study, as summarised above. If exposure ceases at this point, no adverse effects would be expected. **Operator exposure will not exceed 3 months per year.**

The "spray season" for Systhane 20EW is from May to July for vines and April to September for apples. Application to apples early in the season and late in the season will be limited, and at low dose rates. However, Systhane 20EW will never be used for treating vines/apples by any single operator (contractor or farm owner) for >90 consecutive days. A professional sprayer would never spray **continually** in his/her **region** for more than 90 days. Weather (e.g. 90 good spray days) and alternative operator duties would always break the cycle of spraying. Contractors would also have to adhere to some sort of working week. An example of this is shown in an ECPA study which examined the work practice of a number of professional contractors with various products on a selection of crop types during 1995, and covers a period when intense spraying can occur. This study, based on pesticide application records, has shown that application of a Systhane product (containing myclobutanil and sulphur) to vines in southern France would be for a total of 60 hrs/week during a 2 week period (weeks 18 and 19, end April to early May) using an atomiser to treat 100 ha/week (ECPA, 1996). This equates to a total of 20 days on a 6 hour/day basis. Systhane was not used by these contractors at any other time through to end of August, after which records were not presented.

In accordance with the current GAP for Systhane 20EW, a maximum of 4 applications can be made, during the fruit development season. The NOAEL should reflect adverse effects which are expected to occur during this time-frame.
The critical subchronic effects observed with myclobutanil were hepatocellular changes in the 1-year dog study (following 1-year of exposure only) and reproduction effects in the 2-generation rat study.
The NOAEL from the 90-day dog study is 56.8 mg/kg bw/day.
The NOAEL from the 1-year dog study is 14.28 mg/kg bw/day.
The NOAEL from the 2-generation study is 16 mg/kg bw/day.

In the 1-year dog study, changes in ALT were observed from the Week 25 clinical chemistry sample time-point but they did not worsen with increased exposure duration. As the adverse effects (hepatocytes ballooning) in the dog were only seen after one year at 1600 ppm, and not before 3 months (maximum exposure window), the NOAEL from the 2-generation study is appropriate to use for AOEL setting, and would adequately protect against any hepatic or testicular effects of concern.
The use of the 1-year NOAEL from the 2-year chronic rat study is inappropriate as the duration of exposure far exceeds that expected from use of the product. The LOAEL for the testicular effects was 39.2 mg/kg bw/day at 1-year. Similar effects at the 1-year NOAEL of 9.8 mg/kg bw/day were not observed until the 2-year time-point. The 2-generation reproduction study provides a >2-fold margin of safety compared to the 1-year LOAEL.

The appropriate safety factor for setting the AOEL is 100, as there is no justification for using a greater value. The testicular effect is an effect produced from prolonged exposure with a clear NOAEL, and a worker is not going to be exposed to myclobutanil persistently in order for any adverse effects to occur. The 3-month toxicity study in the rat did not show any testicular effects up to and including doses of 585 mg/kg bw/day. The severity of this chronic effect does not warrant an additional safety factor.
In summary, the 2-generation study NOAEL, with a safety factor of 100 gives an AOEL value of **0.16 mg/kg bw/day**.

References:
ECPA. European Agricultural Services SARL. Working patterns of professional field contractors applying agrochemicals. Prepared for ECPA. October 1996.

RMS considers that the proposed AOEL takes into account liver toxicity (and not an adaptative effect) and reproductive toxicity, both effects representing the toxicity profile of myclobutnail.

During Praper 19, it was considered that the dog studies are relevant for setting of the AOEL. An overall NOAEL = 100 ppm (**3.09 mg/kg bw/d)** was proposed with a safety factor of 100 giving an **AOEL =0.03 mg/kg bw/d.**

B.6.12 Dermal absorption (Annex IIIA 7.3)

In the DAR the following proposal was done by the RMS for dermal absorption:

2 *in vivo* dermal absorption studies were provided. Both studies lack informations. According to the applicant, in the first reported study, the total dermal absorption/day was approximately 6% and 11.1% following a 6-hour continuous exposure period for the undiluted and diluted product respectively. From the second study, the total dermal absorption/day was 14% and 9.4% following a 10-hour continuous exposure period for the undiluted and diluted product, respectively.
RMS was not able to understand from where these data were coming from.

RMS proposal: According to the physico-chemical properties, (MW= 288; log Pow=2.556) a default factor of 100% should be used for dermal absorption. The use of a 100% default value seems however to be very conservative.
In the first study, an iv administration was performed in paralel to the dermal administration. The company estimated the bioavailability of myclobutanil by comparing the urinary excretions in both studies. This approach is a realistic estimation of dermal absorption and the RMS will use these results for estimation of operator exposure risk. In this study, dermal absorbed dose within 7 days, after a 6-hour exposure period represents 18% of the concentrate and 30% of the diluted formulation respectively.

The company provided in august 2005 a comparative *in vitro* dermal absorption study using human and rat skin. The results of the study are reported below:

B.6.12.2 Comparative dermal absorption, in vitro using rat and human skin (Annex IIIA 7.3)

- ***In vitro* percutaneous absorption of ^{14}C myclobutanil formulation as an oil in water emulsion (GF-1317) and two field dilutions through rat and human skin (Whittingham, 2005)**

Findings:
In vitro percutaneous absorption of myclobutanil was assessed in rat and human skin membranes at nominal concentrations of 200 g/L, 0.48 g/L and 0.048 g/L, which mimics exposure to the undiluted formulation and concentrations recommended for use in the field. The exposure time was 8 h and post exposure time was 16 h. Prior to application, the solubility of the test compound in the receptor fluid was measured and found to be 0.0379 mg/ml. It can be assumed that the solubility did not limit its absorption. Taking into account the amount of test material in the skin, the mean total absorption was 5.09% (high dose human skin), 5.92% (high dose rat

skin), 17.81% (intermediate dose human skin), 47.66% (intermediate dose rat skin), 21.87% (low dose human skin) and 59.39% (low dose rat skin) (table B.6.12.2-1).

Table B.6.12.2-1 In vitro skin absorption study for an 8 h exposure at three concentrations.

Skin	**Human skin**			**Rat skin**		
Concentration	200 g/L	0.48 g/L	0.048 g/L	200 g/L	0.48 g/L	0.048 g/L
Dose(µg/cm^2)	1875	4.88	0.48	1903	4.86	0.47
N° samples	5	6	5	5	6	6
Recovery of radioactivity after 24 h (% of applied dose)						
Cell wash (donor)	3.06	1.58	4.27	0.87	1.01	0.78
Skin swabs	84.69	79.42	66.26	86.72	51.73	41.15
Tape strips	1.28	6.09	4.05	0.51	1.23	3.07
skin	3.49	5.91	16.09	3.01	8.92	19.74
Receptor fluid + wash	1.60	11.90	5.78	2.92	38.73	39.65
Total recovery	94.12	104.9	96.45	94.03	101.63	104.38
Total absorbed dose *	**5.09**	**17.81**	**21.87**	**5.92**	**47.66**	**59.39**
Penetration into receptor fluid after 24 h:						
% of dose	1.6	11.90	5.78	2.91	38.73	39.65
µg/cm^2	30	0.58	0.028	55.35	1.88	0.19
Absorption rate at steady state (µg/cm^2.h)	1.108	0.016	0.0004	2.039	0.038	0.004

* receptor fluid + receptor wash + skin (excluding tape strips)

Conclusion: taking the results of this *in vitro* skin absorption study, no correction factor has to be applied for the concentrate and a correction factor of 2.7 has to be applied for the diluted test material.

GLP status: yes (no attest of competent authority)
Official protocol: study is not fully conforming OECD test guideline 428 (2000)
Deviation from official protocol: a period of sampling of 24 h is normally required
Material and methods: 6 frozen skin samples from human and rat skin were used. Electrical resistance prior to dosing evaluated skin integrity. Dermatomed skin from human abdomen from cadaver skin and rat skin (thickness was between 450 and 600 µm) were exposed to 6.4 µl of myclobutanil (98.7% purity; batch n° 99-AG-004 (TSN 102721); labeled ^{14}C myclobutanil (specific activity: 6.1 mCi/mmol; radiochemical purity: 99.9%)at low (0.048 g/l, field dilution), intermediate (0.48 g/L, field dilution) or high dose (200 g/L, oil in water emulsion formulation) for a 8-hour period after which each skin membrane was rinsed with 1% tween 80 in water. After the 24 h sample period, the washing was repeated. The receptor fluid consisted of saline (0.9% NaCl) containing sodium azide 0.01% and polyoxyethylene-20-oleyl ether at 6%.

During Praper 19, it was concluded that correction factors of 25% and 15% should be used for dermal absorption for the concentrate and the dilution, respectively.

B.6.15 Exposure data (Annex IIIA 7.2)

B.6.15.1 Estimation of operator exposure (Annex IIIA 7.2.1.1)

The representative use for Systhane 20 EW is presented table B.6.15.1-1. Systhane 20 EW is an emulsion (oil in water) formulation, containing a nominal 200-g/L myclobutanil. It will have one or more applications per crop, per season, at a maximum individual rate of 90 g a.s./ha during the fruit/grain growth/ripening and the maximum duration of the application season will be less than three months. Water is the intended diluent/carrier.
Dermal absorption correction factors: 25% of the concentrate and 15% of the diluted formulation respectively.

Work rate: 8 hectares.
Predicted exposure is compared with the systemic AOEL = 0.03 mg/kg bw/d.
UK POEM and the German model were used to predict exposure to tractor drawn orchard sprayer with hydraulic nozzles (UK model) and tractor high crops (German model) application scenarios. Predicted systemic exposures were calculated and summarized in table B.6.15.1-2.

Applications parameters as proposed by the company:

Table B.6.15.1-1: Application information on representative crops.

Crop	Application method	Max. dose rate L product/ha	Max.dose rate G active substance/ha	Spray volume L/ha	Pack size L
Grape	Air-assisted low and high water volume	0.048	48	1000	1
Apple	Air-assisted low and high water volume	0.09	90	1000	1

Predicted operator exposures made by RMS:

Table B.6.15.1-2: Estimated operator exposure (mg/person/day) for the use of Systhane 20 EW according to the UK POEM model.

Model/Crop/PPEs	Dermal absorbed dose (mg/day)			Inhalation exposure (mg/day)		Total absorbed dose (mg/person/day)
	Mix/load	Spray	Total	Spray	Total	
Orchard low volume Grapes:						
w/o PPEs	0.50	0.10692	0.606	0.001152	0.001152	0.608
With PPEs	0.05	0.0680	0.11804	0.001152	0.001152	0.119192
Orchard low volume Apples						
w/o PPEs	0.5	0.21384	0.713	0.00230	0.00230	0.7161
With PPEs	0.05	0.13608	0.1775	0.00230	0.00230	0.1883

Table B.6.15.1-3: Estimated operator exposure (mg/person/day) for the use of Systhane 20 EW according to the German model.

Model/Crop/PPEs	Estimated exposure mixing /loading (mg/day)		Estimated exposure application (mg/day)		Total exposure mg/day	Total absorbed dose (mg/person/day) cc: 25% and 15% for dil.
	Inhalation	Dermal-hands	Inhal.	Dermal	Total	
Tractor high crop; grapes						
Wo PPEs	0.0002304	0.9216	0.006912	4.4232	5.351	0.8999
With PPEs	0.0002304	0.009216	0.006912	4.157	4.173	0.63192
Tractor high crops; apples						
W/o PPEs	0.000432	1.728	0.01296	8.28	10.02	1.6873
With PPEs	0.000432	0.01728	0.01296	7.798	7.81	1.1848

Comparison of estimated and tolerable exposure:

Table B.6.15.1-3: Exposure as a proportion of AOEL

Crop/application method	Total systemic exposure - 60 kg person (mg/kg bw/day)		% of AOEL	
	No PPE worn	PPE worn	No PPE worn	PPE worn
UK POEM model				
Grapes, orchard, low volume	0.0101	0.00198	34%	6.6%
Apples, orchard, low volume	0.0119	0.00313	39%	10%
German model				
Grapes, orchard	0.01193	0.00902	42%	30%
Apples, orchard	0.02410	0.01692	80%	56%

Conclusions: predicted exposure to myclobutanil formulated as Systhane 20 EW was compared with the systemic AOEL = 0.03mg/kg bw/d. Based upon the exposures predicted, the product can be applied safely with and without PPE for all scenarios according to UK POEM and German model.

B.6.15.2 Measurement of operator exposure (Annex IIIA 7.2.1.2)

No data, not required.

B.6.15.3 Estimation of bystander exposure (Annex IIIA 7.2.2)

In view of the recommended application techniques for Systhane 20 EW, bystanders may be exposed briefly and to relatively low quantities of spray to an operator.

The following assumptions were used in estimating bystander exposure:

1. Maximum applied rate of 90 g a.s./ha at 1000 L spray volume/ha.
2. In a typical case following a single pass of the sprayer, mean potential dermal exposure was measured as 0.1 ml (or 0.0001% of spray volume) of spray on a bystander positioned 8 m from the edge of the treatment area (Lloyd and Bell, 1983). Typical mean potential inhalation exposure was measured as 0.02 ml spray/m^3 (or 0.00001% of spray volume).
3. Body weight is 70 kg.
4. Dermal absorption of spray is 15%
5. Inhalation absorption is 100 %
6. AOEL= 0.03 mg/kg bw/d

Route	Dermal exposure	Inhalation exposure
Volume of spray solution dermally intercepted (ml)	0.1	
Volume of spray solution intercepted by inhalation (ml/m^3)		0.02
Spray volume (L)	1000	1000
Breathing rate (m^3/hour)		3.6
Number of hours worked/day		0.08
Dermal intercepted	0.00005%	
Inhalation intercepted		0.000003%
Application rate (g/ha)	90	90
Amount active intercepted (mg)	0.009	0.0018
Percent absorbed (%)	15%	100%
Absorbed dose (mg)	0.00135	0.0018
Bystander weight (kg)	70	70
Absorbed dose (mg a s/kg bw/d)		0.0000257

Total systemic	0.0000192
	0.0000449
AOEL	0.03 mg/kg bw/d
Exposure % of AOEL:	0.15%

In conclusion, recommended uses of Systhane 20 EW may potentially result in incidental, brief exposure of bystanders to a highly diluted water-based spray drift, but the predicted exposure should be present a negligible risk to their health.

B.6.15.4 Estimation of worker exposure (Annex IIIA 7.2.3.1)

This assessment considers the potential for exposure resulting from the maximum use rate and immediate re-entry, and assumes that PPE is not used.
It covers both workers and non-worker re-entry.
In all re-entry situations, the low volatility of the active substance (1.98×10^{-4} Pa, at 20°C) removes a concern of exposure to vapour. The major route of exposure on re-entry is contact with residues via the skin. The use of the product that represents the greatest concern is on apple and grapes.

Exposure from contact with a treated crop.
Exposure through re-entry into the crop was calculated below for grapes and apples:

Parameters	Value	Reference
Application rate (g/ha)	90	Label
Deposition rate (ng/cm^2 for g a.s./ha)	3	Poppendorf, 1992
Percent dislogeable	80%	Gunther et al., 1973
Max. Dislogeable foliar residue (mg a.s./cm^2)	0.0001152	Calculated (see below)
Body weight	70 kg	
Transfer factor with gloves (cm^2/h)	5000	US EPA RED Diazinon, 2000
Task duration (hour)	8	Assumed
Percent dermal absorption	11%	See dermal absorption studies
Absorbed dose (mg/kg bw/d)	0.00984	Calculated (see below)
AOEL (mg/kg bw/d)	0.03	See proposal for AOEL
Dose as % of AOEL	32%	Calculated (see below)

Where:

Max.dislogeable folair residue= (application rate) x (deposition rate/1000000) x(percent dislogeable/100)

Percent dermal exposure= $\frac{\text{DFR (mg a.s./cm}^2\text{) x transfer coefficient (cm}^2\text{/hr) x task duration (hr/day)}}{\text{Body weight (kg)}}$

In conclusion, even based on the use of maximum theoretical foliar deposits, no intercept by protective clothing and assuming immediate re-entry (at the earliest time associated with GAP), the predicted exposure does not represent an unacceptable risk to human health.

B.6.15.5 Measurement of worker exposure (Annex IIIA 7.2.3.2)

Not necessary, not required.

B.6.16 References relied on

Toxicology and metabolism of the active substance (Annex II A 5)

Annex point/ref number	**Authors**	**Year**	**Title** **Testing facility** **Owner/source/where different from owner** **Report n°** **GLP or GEP status (where relevant)** **Published or not**	**Data protection claimed Yes/No**	**OWNER**
IIA 7.3	Whittingham, A	August 2005	*In vitro* percutaneous absorption of ^{14}C myclobutanil formulation as an oil in water emulsion (GF-1317) and two field dilutions through rat and human skin Dow Agro Sciences Company GLP status: yes Published: no	?	Dow Agro Sci

ANNEX B

Myclobutanil

Appendix: New estimation of the exposure

UK POEM: tractor drawn assisted orchard sprayer 100 L/ha model- grapes

Product data

Product	Systhane 20 EW
Active substance	myclobutanil
Concentration	200 mg/ml
Formulation type	EC
Maximum in use a.s.concentration	0.0096 mg/ml

Exposure during mixing and loading

Container size	1 L
Hand contamination/operation	0.01 ml
Application dose	0.048L product/ha
Work rate	8 ha/day
Number of operations	1 day
Hand contamination	0.01 g/day
Protective clothing	None
Transmission to skin	100%
Dermal exposure to formulation	0. 01 g/day

Exposure during spray application

Application technique-tractor drawn orchard sprayer with hydraulic nozzles

Application volume	1000 spray/ha		
Volume of surface contamination	50 ml/h		
Distribution	Hands	Trunk	Leggs
	10	65	25%
Clothing	None	Permeable	Permeable
	100	15	20%
Dermal exposure	5	4.875	2.5 ml/h
Duration of exposure	6 h		
Total dermal exposure to spray	74.25 ml/day		

Absorbed dose	Mix/load	Mix/load
Dermal exposure	0.01 ml/day	74.25 ml/day
Concentration of a.s.	200 mg/ml	0.0096 mg/ml
Dermal exposure to a.s.	2 mg/day	0.7128 mg/day
Percent absorbed	25%	15%
Absorbed dose	050 mg/day	0.1069mg/day

Inhalation exposure during spraying

Inhalation exposure	0.02 ml/h
Duration of exposure	6 h
Concentration of a.s.	0.0096mg/ml
Inhalation exposure to a.s.	0.001152 mg/day
Percent absorbed	100%
Absorbed dose	0.001152 mg/day

Predicted exposure

Total absorbed dose	0.608 mg/day
Operator exposure weight	60 kg

Operator exposure	0.0101 mg/kg bw/d

UK POEM: tractor drawn assisted orchard sprayer 100 L/ha model- grapes with PPEs

Product data

Product	Systhane 20 EW
Active substance	myclobutanil
Concentration	200 mg/ml
Formulation type	EC
Maximum in use a.s.concentration	0.0096 mg/ml

Exposure during mixing and loading

Container size	1 L
Hand contamination/operation	0.01 ml
Application dose	0.048L product/ha
Work rate	8 ha/day
Number of operations	1 day
Hand contamination	0.01 g/day
Protective clothing	Gloves
Transmission to skin	100%
Dermal exposure to formulation	0. 001 g/day

Exposure during spray application

Application technique-tractor drawn orchard sprayer with hydraulic nozzles

Application volume	1000 L spray/ha		
Volume of surface contamination	50 ml/h		
Distribution	Hands	Trunk	Leggs
	10	65	25%
Clothing	Gloves	Permeable	Permeable
	10	15	20%
Dermal exposure	0.5	4.875	2.5 ml/h
Duration of exposure	6 h		
Total dermal exposure to spray	47.25 ml/day		

Absorbed dose	Mix/load	Mix/load
Dermal exposure	0.001 ml/day	47.25 ml/day
Concentration of a.s.	200 mg/ml	0.0096 mg/ml
Dermal exposure to a.s.	0.2mg/day	0.4536 mg/day
Percent absorbed	25%	15%
Absorbed dose	0.05 mg/day	0.068 mg/day

Inhalation exposure during spraying

Inhalation exposure	0.02 ml/h
Duration of exposure	6 h
Concentration of a.s.	0.0096 mg/ml
Inhalation exposure to a.s.	0.0001152mg/day
Percent absorbed	100%
Absorbed dose	0.001152 mg/day

Predicted exposure

Total absorbed dose	0.1192mg/day
Operator exposure weight	60 kg
Operator exposure	0.00198 mg/kg bw/d

UK POEM: tractor drawn assisted orchard sprayer 100 L/ha model- apples

Product data

Product	Systhane 20 EW
Active substance	myclobutanil
Concentration	200 mg/ml
Formulation type	EC
Maximum in use a.s.concentration	0.018 mg/ml

Exposure during mixing and loading

Container size	1 L
Hand contamination/operation	0.01 ml
Application dose	0.09 L product/ha
Work rate	8 ha/day
Number of operations	1 day
Hand contamination	0.01 g/day
Protective clothing	None
Transmission to skin	100%
Dermal exposure to formulation	0. 01g/day

Exposure during spray application

Application technique-tractor drawn orchard sprayer with hydraulic nozzles

Application volume	1000 L spray/ha		
Volume of surface contamination	50 ml/h		
Distribution	Hands	Trunk	Leggs
	10	65	25%
Clothing	None	Permeable	Permeable
	100	15	20%
Dermal exposure	5	4.875	2.5 ml/h
Duration of exposure	6 h		
Total dermal exposure to spray	74.25 ml/day		

Absorbed dose	Mix/load	Mix/load
Dermal exposure	0.01 ml/day	74.25 ml/day
Concentration of a.s.	200 mg/ml	0.018 mg/ml
Dermal exposure to a.s.	2 mg/day	1.3365 mg/day
Percent absorbed	25%	15%
Absorbed dose	0.5 mg/day	0.2004 mg/day

Inhalation exposure during spraying

Inhalation exposure	0.02 ml/h
Duration of exposure	6 h
Concentration of a.s.	0.018 mg/ml
Inhalation exposure to a.s.	0.00216 mg/day
Percent absorbed	100%
Absorbed dose	0.00216 mg/day

Predicted exposure

Total absorbed dose	0.7026 mg/day
Operator exposure weight	60 kg
Operator exposure	0.0117 mg/kg bw/d

UK POEM: tractor drawn assisted orchard sprayer 500 L/ha model- apples with PPEs

Product data

Product	Systhane 20 EW
Active substance	myclobutanil
Concentration	200 mg/ml
Formulation type	EC
Maximum in use a.s.concentration	0.018 mg/ml

Exposure during mixing and loading

Container size	1 L
Hand contamination/operation	0.01 ml
Application dose	0.09 L product/ha
Work rate	8 ha/day
Number of operations	1 day
Hand contamination	0.01 g/day
Protective clothing	Gloves
Transmission to skin	10%
Dermal exposure to formulation	0. 001 g/day

Exposure during spray application

Application technique-tractor drawn orchard sprayer with hydraulic nozzles

Application volume	1000 L spray/ha		
Volume of surface contamination	50 ml/h		
Distribution	Hands	Trunk	Leggs
	10	65	25%
Clothing	Gloves	Permeable	Permeable
	10	15	20%
Dermal exposure	0.5	4.875	2.5 ml/h
Duration of exposure	6 h		
Total dermal exposure to spray	47.25 ml/day		

Absorbed dose	Mix/load	Mix/load
Dermal exposure	0.001 ml/day	47.25 ml/day
Concentration of a.s.	200 mg/ml	0.018 mg/ml
Dermal exposure to a.s.	0.2 mg/day	0.8505 mg/day
Percent absorbed	25%	15%
Absorbed dose	0.05 mg/day	0.1275 mg/day

Inhalation exposure during spraying

Inhalation exposure	0.02 ml/h
Duration of exposure	6 h
Concentration of a.s.	0.018 mg/ml
Inhalation exposure to a.s.	0.00216 mg/day
Percent absorbed	100%
Absorbed dose	0.00216 mg/day

Predicted exposure

Total absorbed dose	0.1797mg/day
Operator exposure weight	60 kg
Operator exposure	0.00299 mg/kg bw/d

German model: wine grape

Use information				
Product	Systhane 20EW	Active substance	myclobutanil	
Formulation type	liquid	a.s. concentration	200 mg/ml	
Method of use	Tractor high crops	Dose(product)	1000 L product/ha	
Work rate	8 ha/day	Dose (a.s.)	0.048 kg a.s./ha	
		Amount handled	0.384 kg a.s./day	
Exposures-mix/loading				
	Specific exposures	Estimated exposures	PPE	Estimated exposures
Inhalation	0.0006 mg/kg a.s.handled	0.0002304 mg a.s./day	None	0.0002304 mg a.s./day
Dermal-hands	2.4 mg/kg a.s.handled	0.9216 mg a.s./day	Gloves	0.009216 mg a.s./day

Exposures-application

	Specific exposures	Estimated exposures	PPE	Estimated exposures (PPE)
Inhalation	0.018 mg/kg a.s.handled	0.006912 mg a.s./day	None	0.006912 mg a.s./day
Dermal-head	1.2 mg/kg a.s.handled	0.4608 mg a.s./day	None	0.4608 mg a.s./day
Dermal –hands	0.7 mg/kg a.s.handled	0.2688 mg a.s./day	Gloves	0.002688 mg a.s./day
Dermal- body	9.6 mg/kg a.s.handled	3.6864 mg a.s./day	none	3.6864 mg a.s./day
Total exposures		Estimated exposures	Percent absorbed	Estimated exposures (PPE)
Total potential inhalation		0.007142 mg a.s./day	100%	0.00714 mg a.s./day
Total dermal-mix		0.9216 mg a.s./day	25%	0.009216 mg a.s./day
Total dermal-application		4.416 mg a.s./day	15%	4.149 mg a.s./day
Total absorbed dose		0.8999 mg a.s./day		0.6319 mg a.s./day
Body weight		70 kg		70 kg
Mg/kg bw/d		0.01285 mg/kg bw/d		0.00902 mg/kg bw/d

German model: apple

Use information				
Product	Systhane 20EW	Active substance	myclobutanil	
Formulation type	liquid	a.s. concentration	200 mg/ml	
Method of use	Tractor high crops	Dose(product)	1000 L product/ha	
Work rate	8 ha/day	Dose (a.s.)	0.09 kg a.s./ha	
		Amount handled	0.72 kg a.s./day	
Exposures-mix/loading				
	Specific exposures	Estimated exposures	PPE	Estimated exposures
Inhalation	0.0006 mg/kg a.s.handled	0.000432mg a.s./day	None	0.000432 mg a.s./day
Dermal-hands	2.4 mg/kg a.s.handled	1.728 mg a.s./day	Gloves	0.01728 mg a.s./day

Exposures-application

	Specific exposures	Estimated exposures	PPE	Estimated exposures (PPE)
Inhalation	0.018 mg/kg a.s.handled	0.01296 mg a.s./day	None	0.01296 mg a.s./day
Dermal-head	1.2 mg/kg a.s.handled	0.864 mg a.s./day	None	0.864 mg a.s./day
Dermal –hands	0.7 mg/kg a.s.handled	0.504 mg a.s./day	Gloves	0.00504 mg a.s./day
Dermal- body	9.6 mg/kg a.s.handled	6.912 mg a.s./day	none	6.912 mg a.s./day
Total exposures		Estimated exposures	Percent absorbed	Estimated exposures (PPE)
Total potential inhalation		0.01339 mg a.s./day	100%	0.01339 mg a.s./day
Total dermal-mix		1.728 mg a.s./day	25%	0.01728 mg a.s./day
Total dermal-application		8.28 mg a.s./day	15%	7.781 mg a.s./day
Total absorbed dose		1.6873mg a.s./day		1.1848 mg a.s./day
Body weight		70 kg		70 kg
Mg/kg bw/d		0.0241 mg/kg bw/d		0.01692 mg/kg bw/d

ANNEX B

Myclobutanil

B.7 Residue data
(Addendum March 2007)

Open point 3.1 :

-The metabolism of RH-3866 in Apples (Nelson S.S., Streelman D.R.; 1984c)

Extraction procedure :
*Juice :
Juice was neutralized by addition of 10 mL of NaHCO3 and was diluted with water. The diluted juice was extracted with chloroform (3 x) and the combined were evaporated to dryness. The aqueous phase was further extracted with n-butanol (3 x) and the extracted fractions were also taken to dryness.
*Pomace :
Pomace was extracted with refluxing methanol and the methanolic extract was reduced to dryness and taken up in water. The following extraction steps were similar as for juice, i.e., the aqueous sample was further partitioned with chloroform (3 x) and the combined were evaporated to dryness. The aqueous phase was further partitioned with n-butanol (3 x) and the extracted fractions were also taken to dryness.

Both for juice and pomace, the dried butanol fractions were then dissolved in acidified methanol in order to attempt to hydrolyse any conjugates present. The evaporated hydrolysis mixture was re-suspended in water and then partitioned against chloroform to provide the organo soluble and water soluble partitioned phases.
Both the original chloroform extract and the chloroform extracts from the hydrolysis of the butanol fractions were analysed by TLC analysis for the metabolites identification by chromatographic comparison with reference standards.
*Whole fruit : None.
The total radioactive residues in the whole fruit were calculated considering the mass balance between juice and pomace.

Findings :
Table B.7.1.2-1 : Investigation of the nature and the amounts of residues of (Phenyl ring –^{14}C)-myclobutanil and (Triazole ring –^{14}C)-myclobutanil in/on apple trees at harvest following 10 weekly spray treatments to run-off each at a field rate of 240 g a.s./ha (Residues expressed as mg myclobutanil equiv./kg and in % of the total radioactive residues).

Test substances	**(Phenyl ring-^{14}C)- myclobutanil**			**(Triazole ring -^{14}C)-myclobutanil**		
Sample	**Whole fruit[(1)]**	**Juice**	**Pomace**	**Whole fruit[(1)]**	**Juice**	**Pomace**
Total radioactive residues (mg myclobutanil equiv./kg)						
	0.48[(1)]	0.15	1.00	0.32[(1)]	0.12	0.66
Extractability of the total radioactivity - % of the TRR and *(mg myclobutanil equivalent/kg)*						
Methanol extraction phase		np	*88.0 (0.88)[(2)]*		np	*90.9 (0.60)[(2)]*
Chloroform extraction phase		52.2 *(0.078)*	73.7 *(0.648)*		53.5 *(0.064)*	73.9 *(0.443)*
~~Organosoluble 1~~ n-butanol ~~partitioned~~ extraction phase		44.2 *(0.066)*	24.5 *(0.215)*		33.5 *(0.0402)*	22.1 *(0.132)*
Chloroform partitioned phase (after acid hydrolysis)		89.0 *(0.058)*	93.5 *(0.201)*		82.8 *(0.033)*	90.6 *(0.119)*
Water partitioned phase (after acid hydrolysis)		11.0 *(0.0072)*	6.5 *(0.0139)*		17.2 *(0.0069)*	9.4 *(0.0124)*
Water soluble phase (remaining aqueous phase fraction after the successive extractions)		8.3 *(0.012)*	1.8 *(0.0158)*		12.8 *(0.015)*	4.0 *(0.024)*
Elucidation of the radioactive residues - % of the TRR and *(mg myclobutanil equivalent/kg)*						
Parent RH-3866	48.5 *(0.232)*	21.7 *(0.032)*	54.9 *(0.549)*	48.7 *(0.155)*	23.8 *(0.028)*	56 *(0.369)*
RH-9089	1.9 *(0.0091)*	1.3 *(0.008)*	1.9 *(0.019)*	2.9 *(0.0092)*	1.2 *(0.0014)*	3.4 *(0.022)*
RH-9090	11.5 *(0.055)*	26.5 *(0.039)*	7.9 *(0.079)*	11.5 *(0.0368)*	24.7 *(0.029)*	7.6 *(0.05)*

Test substances	(Phenyl ring-^{14}C)- myclobutanil			(Triazole ring -^{14}C)-myclobutanil		
Sample	**Whole fruit(1)**	**Juice**	**Pomace**	**Whole fruit(1)**	**Juice**	**Pomace**
RH_9090 glucoside	23.7 *(0.0113)*	40.7 *(0.061)*	19.7 *(0.197)*	20.9 *(0.066)*	30.0 *(0.036)*	18.3 *(0.12)*
Total identified metabolites	85.5 *(0.41)*	90.2 *(0.135)*	84.4 *(0.844)*	84 *(0.268)*	79.7 *(0.095)*	85.3 *(0.562)*
Residual radioactive residues - % of the TRR and *(mg myclobutanil equivalent/kg)*						
		Not relevant	10.5 (0.105)		Not relevant	8.9 (0.058)
Accountability : partitioned phases + residual radioactive residues						
		104.7	~~100.0~~ 98.5		99.8	~~100.0~~ 99.8

np : not performed
(1): The residues in the whole fruit were calculated from residues in juice and pomace fractions.
The extraction procedure for the whole fruit was not proposed.
(2) : Since 25 g of pomace were extracted and the amount of radioactivity recovered in the pomace after the extractions is available, we can calculate the amount of radioactivity in the starting methanol extracts. The detailed calculation is given here below.
Remark :
The majority of the radioactivity recovered in the hydrolysed fractions was the metabolite RH-9090.
The metabolites distribution differs between the juice and the pomace with the juice containing a higher percentage of the more water soluble RH-9090 (free/conjugated forms) while the pomace concentrated more the parent compound.

	Phenyl Label	**Triazole Label**
Pomace (by combustion)	1.0 ppm	0.66 ppm
Specific Activity	4410 dpm/µg	4380 dpm/µg
25 g pomace (used for Methanol soxhlet extraction)	110,250 dpm calculated	72,664 dpm calculated
Methanol Extracted Pomace Residue (by combustion)	13,680 dpm	7,320 dpm
Difference between starting pomace (dpm) and extracted pomace (dpm)	110,250 dpm – 13,680 dpm = 96,570 dpm in Methanol	72,664 dpm – 7,320 dpm = 65,344 dpm in Methanol
Amount of Radioactivity in Methanol from Pomace	96,570 dpm	65,344 dpm
TRR (based on 25 g subsample)	0.88 ppm	0.60 ppm
Remark : Residues (ppm)=total dpm/specific activity x sample weight		

At harvest, the total radioactive residues in/on whole fruit of apple amounted about 0.48 and 0.32 mg myclobutanil equivalents /kg respectively for the phenyl ring and the triazole ring labelling forms.
Residue levels in juice were much lower than those in pomace and whole fruit with an average of 0.14 mg/kg for both phenyl and triazole labelled samples.
About 20 % of the total residues were transferred into juice at processing.

~~More than 52 % and 73 % of the TRR could be extracted from juice and pomace respectively for both the 2 labelling forms~~. ~~After liquid/liquid partitioning~~ After successive extraction steps, the extractable radioactivity was predominantly found in the ~~organosoluble~~ chloroform phase for juice and pomace (~~44~~ 52 % and ~~33~~ 73 % of the TRR respectively for the phenyl and the triazole ring label forms). ~~A similar extractability pattern was observed in the pomace with up to 24 % of the TRR.~~
~~The whole fruit was not submitted to the extractability pattern and the total residues were calculated from juice and pomace fractions.~~
~~In consequence~~, the results for the whole fruit were determined by combining the results for the juice and pomace based on a proportional evaluation of the data.
Around 50 % of the total residues could be expected to be the parent compound.
Conjugated RH-9090 accounted for up to 22 % of the TRR and to a minor extent to the free RH-9090 with 11.5% of the TRR for both the 2 labelling forms.

In pomace, myclobutanil represented the major compound of the total residues with up to 56 % of the TRR for both the 2 labelling forms.
In juice, the parent compound was also present in non negligible amounts (up to 23.8 % of the TRR for the 2 labellings) but was also extensively metabolized into organo soluble metabolites identified primarily as the alcohol RH-9090 (up to 26.5 % of TRR), its glucose conjugate (up to 40 % of TRR) and the minor ketone metabolite RH-9089 (1.3 % of TRR).
It was shown that there was no marked change in the nature and the amount of radioactive residues during sample storage over a time period of 24 months for apples.

Conclusion :
The distribution of the metabolites didn't differ significantly with the labelling form indicating that no significant cleavage of the phenethyl triazole linkage occurred.
Myclobutanil is the major constituant of the residues in/on apples and pomace 14 days after the final application and still constitutes a valid indicator of the residue level in juice.
The main metabolic transformation of myclobutanil in apples initially involves oxidation of the butyl group of the parent molecule into the alcohol derivate metabolite RH-9090 and then a conjugation reaction phase to give the conjugated RH-9090 glucoside.
An other minor route consisted of further oxidation of the metabolite RH-9090 to generate the minor ketone metabolite RH-9089.

Open point 3.2 :

-Laboratory Metabolism Studies of ^{14}C-RH-3866 in Wheat –Report TR 310-84-10 (Nelson S.S., 1984a)
Guidelines :
Not specified.
GLP :
No. Not required at the time the study was conducted.

Material and Methods :
Test substances : (Phenyl ring –^{14}C)-myclobutanil and (Triazole ring –^{14}C)-myclobutanil.
Experimental design :
The studies were carried out under greenhouse conditions.
3 experiments were performed using wheat seedlings, freshly excised wheat shoots and freshly excised wheat heads.
-In the excised wheat shoots study, the shoots were transferred from water into a solution containing a nutrient solution and either the phenyl ring or the triazole ring labelling form for myclobutanil uptake for 1 day and 5 days. The concentrations of the test substances in the uptake solutions varied according to the uptake period (for the 1 day experiment, the uptake solution contained 140 ppm and 136 ppm respectively for the phenyl ring and the triazole ring labelling forms. For the 5 days experiment, the concentrations used were 47 ppm and 35 ppm respectively for the phenyl and triazole ring labelling forms).
At the end of the experiment, the shoots ends were washed either with methanol or with water to remove any residues of myclobutanil.
-In the wheat seedlings experiment, the seedlings were grown hydroponically and transferred from water into a nutrient solution containing also 42 ppm and 64 ppm of phenyl ring and triazole ring labelling forms respectively. After 11 days of experimentation, the plants roots were rinsed with water to wash any residues of myclobutanil on the root surface.
-In the third experiment, excised wheat heads were placed in an uptake solution containing nutrients and either the test substances at concentrations of 17 ppm or 11 ppm respectively for the phenyl and triazole ring labelled forms for 13 days
Extraction procedure :
Radioactivity contained in the different solid and liquid crop fractions was measured by radiocombustion analysis followed by Liquid Scintillation Counting (LSC).
The different wheat plant parts were extracted with methanol followed by liquid/liquid partitioning against hexane to give organic and aqueous phases.
The water soluble residues of the different plant parts were subsequently submitted to liquid/liquid partitioning against various polar solvents (methylene chloride, ethyl acetate, 1-butanol and chloroform).
Purification of the metabolites fractions was performed by preparative 1D-TLC analysis.

Identification of the metabolites in the excised wheat shoots extracts was performed using GC/ECD by chromatographic comparison with reference compounds.
The wheat seedlings and the excised wheat heads extracts were characterized using 1D-TLC analysis.
The chemical structure of some metabolites was elucidated by GC/Mass spectrometry analysis.

Findings :
B.7.1.3-1 : Investigation of the amounts and the nature of the residues of (Phenyl ring –^{14}C)-myclobutanil and (Triazole ring –^{14}C)-myclobutanil in excised wheat shoots, in intact wheat seedlings and in excised wheat heads after a determined uptake period of the test substances (Residues expressed in percent of total radioactive residues).

Test substances	**(Phenyl ring –^{14}C)-myclobutanil**				**(Triazole ring –^{14}C)-myclobutanil**			
Sample	**Excised wheat shoots**		**Wheat seedlings**	**Wheat heads**	**Excised wheat shoots**		**Wheat seedlings**	**Wheat heads**
Uptake period	**1 day**	**5 days**	**11 days**	**13 days**	**1 day**	**5 days**	**11 days**	**13 days**
Total radioactive residues (% of the TRR)								
	Not provided.							
Extractability of the total radioactivity (% of the TRR)								
Methanol extracted phase	Not provided.							
Organosoluble hexane partitioned phase	1.9	1.2	1.0	18.3	1.8	1.2	0.9	6.0
Organosoluble Methylene chloride partitioned phase	94.6	81.9	60.6	NA	95.2	81.0	69.8	NA
Organosoluble ethyl acetate partitioned phase	1.7	NA	NA	NA	1.8	NA	NA	NA
Organosoluble 1-butanol partitioned phase	NA	11.7	22.8	23.5	NA	12.9	16.9	19.7
Organosoluble chloroform partitioned phase	NA	NA	NA	55.9	NA	NA	NA	72.9
Aqueous soluble phase	1.7	2.3	7.3	1.2	1.2	2.4	4.7	0.5
Soxhlet extract	NA	2.4	6.7	NA	NA	2.0	6.4	NA
Elucidation of the total radioactive residues (% of the TRR)								
Parent RH-3866	-	73	62	73	-	72	71	75
Alcohol RH-9090	-	6	2	5	-	6	2	4
Alcohol RH-9090 glucoside	-	5	15	16	-	7	11	18
RH 9090-malonyl glucoside(1)	-	5	15	ND	-	5	10	ND
Unknown compounds	-	0.5	2	1	-	0.4	1	1
Total identified metabolites	-	89.5	96.0	95.0	-	90.4	95.0	98.0
Residual radioactive residues (% of the TRR)								
		0.5	1.6	1.1		0.4	1.3	0.8
Accountability : partitioned phases + residual radioactive residues								
	99.9	100.0	100.0	100.0	100.0	99.9	100.0	99.9

NA : not applicable
-: Not analysed.
ND : not radiodetected.
(1) : The identity of this metabolite couldn't be confirmed since it is rather unstable as to be expected because malonic esters are easily hydrolysed.

In the wheat seedlings, most of the radioactivity (62 to 71 % of the TRR) remained as unchanged parent compound and the total conjugated forms of the alcohol RH-9090 (glucoside and malonyl glucoside) constituted the complement of the total residues (accounting for 30 % of the TRR and 21 % of the TRR for the phenyl and the triazole ring labelling forms respectively).
In the excised wheat shoots, more than 72 % of the TRR remained as intact myclobutanil.
The residues in the day 13 uptake excised heads were constituted of the parent compound (with up to 75 % of the TRR). The alcohol metabolite RH-9090 accounted for 5 % of the TRR and the amount of glucoside conjugate of RH-9090 raised 18 % of the TRR for both the 2 labelling forms.
Conclusion :

The major part of the total residue was represented by the parent myclobutanil along with the glucose and the malonyl glucose conjugates of the alcohol metabolite RH-9090 as non negligible metabolites.
This study is only indicative and cannot therefore be reliable for the evaluation of the metabolism of Myclobutanil in wheat.

Open point 3.5 : The reference should be deleted in the list of studies relied on.

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not
IIA 6.0/01	Deakyne, R.O., Brackett, C.K, Stavinski, S.S., Burnett, T.F.	1986a	RH-3866 Storage Stability Study in Apples. Rohm and Haas Company. DAS Report No.: 31H-86-04 (Masterfile Number) ER R84.4 GLP/GEP (Y/N): N Published (Y/N): N
IIA 6.0/02	Deakyne, R.O., Brackett, C.K., Burnett, T.F., Stavinski, S.S.	1986b	RH-3866 Storage Stability Study in Grapes. Rohm and Haas Company. DAS Report No.: 31H-86-06 (Masterfile Number) ER R83.4 GLP/GEP (Y/N): N Published (Y/N): N
IIA 6.0/03	Batra, R.	1997a	Storage Stability Study : RH-3866 (myclobutanil fungicide) & RH-9090 in Almond Meat and Hulls. Rohm and Haas Company / QC, Inc. DAS Report No.: TR 34-96-155 (Masterfile Number) ER R106.1 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.0/04	Batra, R.	1995	Storage stability Study : RH-3866 & RH-9090 in Cucurbits. Rohm and Haas Company / Centre Analytical Laboratories. DAS Report No.: 34A-94-30 (Masterfile Number) ER R91.3 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.0/05	Batra, R.	1997b	Storage Stability Study : RH-3866 & RH-9090 in Tomatoes. Rohm and Haas Company / Centre Analytical Laboratories, Inc. DAS Report No.: 34-96-157 (Masterfile Number) ER R102.5 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.0/06	Cui, Y.	1997	RH-3866 and RH-9090 Storage stability in liver and muscle

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not
			Rohm & Haas Company/Centre Analytical Labs DAS Report No.: Derbi 94239; TR 34-97-118 (Masterfile Number) ER 59.8 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.0/07	Desai, R.; Garstka, T.A.	1997	RH-80294 Storage stability in milk DAS DAS Report No.: Derbi 94171; TR 34-97-117 (Masterfile Number) ER 59.9 GLP/GEP (Y/N): Y Published (Y/N): N
~~IIA 6.1/01~~	~~Nelson, S.S.~~	~~1984a~~	~~Laboratory Metabolism Studies of 14C RH-3866 in Grapes.~~ ~~Rohm and Haas Company.~~ ~~DAS Report No.: 31O-84-15~~ ~~(Masterfile Number) ER 13.1~~ ~~GLP/GEP (Y/N): N~~ ~~Published (Y/N): N~~
IIA 6.1/02	Nelson, S.S.	1984b	Metabolism of 14C RH-3866 in Field Treated Grapes. Rohm and Haas Company. DAS Report No.: 31O-84-30 (Masterfile Number) ER 15.2 GLP/GEP (Y/N): N Published (Y/N): N
IIA 6.1/03	Nelson, S.S., Streelman, D.R.	1984c	The Metabolism of RH-3866 in Apples. Rohm and Haas Company. DAS Report No.: 31O-84-31 (Masterfile Number) ER 15.1 GLP/GEP (Y/N): N Published (Y/N): N
~~IIA 6.1/04~~	~~Nelson, S.S.~~	~~1984a~~	~~Laboratory Metabolism Studies of 14C RH-3866 in Wheat.~~ ~~Rohm and Haas Company.~~ ~~DAS Report No.: 31O-84-10~~ ~~(Masterfile Number) ER 39.2~~ ~~GLP/GEP (Y/N): N~~ ~~Published (Y/N): N~~
IIA 6.1/05	Streelman, D.R.	1984	The Metabolism of RH-3866 in wheat. Rohm and Haas Company. DAS Report No.: 31O-84-17 (Masterfile Number) ER 39.6 GLP/GEP (Y/N): N Published (Y/N): N
~~IIA 6.2/01~~	~~Jacobson, A.~~	~~1986a~~	14C RH-3866 Feeding Study in Cows.

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not
			Rohm and Haas Company. DAS Report No.: 31H-86-13 (Masterfile Number) ER 23.9 GLP/GEP (Y/N): N Published (Y/N): N
IIA 6.2/02	Jacobson, A.H.	1986b	Characterization and Identification of Metabolites in Cows Fed a 14C Mixture of RH-3866/RH-9090/RH-9089. [redacted] DAS Report No.: 31H-86-18 (Masterfile Number) ER 23.10 GLP/GEP (Y/N): N Published (Y/N): N
IIA 6.2/03	Jacobson, A.	1986bc	14C RH-3866 Feeding Study in Poultry. [redacted] DAS Report No.: 31H-86-16 (Masterfile Number) ER 21.6 GLP/GEP (Y/N): N Published (Y/N): N
IIA 6.2/04	Martin, J.J.	1986	Disposition and Metabolism of RH-3866 and Metabolites in Laying Hens. [redacted] DAS Report No.: 31H-86-17 (Masterfile Number) ER 21.7 GLP/GEP (Y/N): N Published (Y/N): N
IIA 6.3.1/01	Gilbert, J.	1997a	To Determine the Magnitude of Res. of myclobutanil and the Metabolite RH-9090 during the 14 days following the Final Application in the Raw Ag. Commodity of Apples Resulting from Sequential Directed Application of Systhane 20EW in Germany. Huntingdon Life Sciences Ltd. DAS Report No.: R&H 205/971531 (Masterfile Number) ER R96.5 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.3.1/02	Gilbert, J.	1998a	To Determine The Magnitude of Residues During the 14 days Following the Final Application in the Raw Ag. Commodity of Apples Resulting from Sequential Directed Application of Systhane 20EW in the UK. Huntingdon Life Sciences Ltd. DAS Report No.: RAS 11/982572 (Masterfile Number) ER R100.1 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.3.1/03	Distler, B.	1993a	Myclobutanil Apple Residue Studies 1986. Dr Specht Laboratories.

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not
			DAS Report No.: DEU86F21211 to DEU86F21241 (Masterfile Number) ER R75.14 GLP/GEP (Y/N): N Published (Y/N): N
IIA 6.3.1/04	Gilbert, J.	1997b	To Determine the Magnitude of Residues of myclobutanil and the Metabolite RH-9090 During the 14 Days Following the Final Application in the Raw Ag. Commodity of Apples Resulting from Sequential Directed Application of Syst 20EW in Europe. Huntingdon Life Sciences Ltd. DAS Report No.: R&H 216/971976 (Masterfile Number) ER R97.1 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.3.1/05	Gilbert, J.	1997c	To Determine the Magnitude of Residues of myclobutanil and the Metabolite RH-9090 During the 14 days Following the Final Application in the Raw Ag. Commodity of Apples Resulting from Sequential Directed Application of Systhane 12E in Europe Huntingdon Life Sciences Ltd. DAS Report No.: R&H 215/972166 (Masterfile Number) ER R96.8 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.3.1/06	Gilbert, J.	1998b	To Determine the Magnitude of Residues during the 14 days Following the Final Application in the Raw Ag. Commodity of Apples Resulting from Sequential Directed Application of Systhane 20EW in Italy and Greece. Huntingdon Life Sciences Ltd. DAS Report No.: RAS 21/974499 (Masterfile Number) ER R100.4 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.3.1/07	Maigrot, P.	1994	Determination of the Residues of Myclobuatnil and its Metabolites in Apples in Spain, 1993 Anadiag S.A DAS Report No.: Derbi 138353; RF 311003 (Masterfile Number) ER R86.12 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.3.1/08	Gilbert, J.	1997d	To Determine the Magnitude of Residues of Myclobutanil and the Metbaolite RH-9090 During the 14 Days Following the Final Application in the Raw Agricultural Commodity of Apples Resulting from Sequential Directed Application of Systhane 24E Huntingdon Life Sciences Ltd.

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not
			DAS Report No.: Derbi 138355; R&H 214 (Masterfile Number) ER R96.7 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.3.1/09	Gilbert, J.	1997e	To Determine the Magnitude of Residues of Myclobutanil and the Metbaolite RH-9090 During the 14 Days Following the Final Application in the Raw Agricultural Commodity of Apples Resulting from Sequential Directed Application of Systhane Flo Huntingdon Life Sciences Ltd. DAS Report No.: Derbi 137145; R&H 217 (Masterfile Number) ER R97.2 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.3.1/10	Oxspring, S.	2005a	To Determine the Magnitude of Myclobutanil Residues at Harvest and at Intervals in the Raw Agricultural Commodity Apples and Processed Fractions Resulting from Sequential Overall Applications of GF-1317, in Northern France, 2004 Agrisearch UK Ltd DAS Report No.: GHE-P-10967 (Masterfile Number) ER R 106.9 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.3.1/11	Oxspring, S.	2005b	To Determine the Magnitude of Myclobutanil Residues at Harvest and at Intervals in the Raw Agricultural Commodity Apples Resulting from Sequential Overall Applications of GF-1317, in Southern France and Spain, 2004 Agrisearch UK Ltd DAS Report No.: GHE-P-10964 (Masterfile Number) ER R 106.10 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.3.2/01	Gilbert, J.	1997f	To Determine the Magnitude of Residues of myclobutanil & RH-9090 During the 28 days Following the Final Application in the Raw and Processed Ag. Commodity of Grapes Resulting from Sequential Directed Application of Systhane 20EW in Germany. Huntingdon Life Sciences Ltd. DAS Report No.: R&H 203/971083 (Masterfile Number) ER R95.4 GLP/GEP (Y/N): Y Published (Y/N): N

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not
IIA 6.3.2/02	Feilden, A.	1998	To Determine the Magnitude of Residues During the 28 Days Following the Final Application in the Raw Agricultural and Processed Commodity of Wine Grapes Resulting from Sequential Directed Application of Systhane 20EW in Northern France. Huntingdon Life Sciences Ltd. DAS Report No.: TR-34-98-43; RAS 18/980226 (Masterfile Number) ER R101.2 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.3.2/03	Gilbert, J.	1997g	Residues in grapes - Germany Huntingdon Life Sciences Ltd. DAS Report No.: Derbi 94404; R&H 202 (Masterfile Number) ER 96.2 GLP/GEP (Y/N): Y Published (Y/N): N

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not
IIA 6.3.2/04	Gilbert, J.	1997h	To Determine the Magnitude of Residues of Myclobutanil and Metabolite RH-9090 During the 14 Days Following the Final Application in the Raw Ag. Commodity of Wine Grapes Resulting from Sequential Directed Application of Syst 20EW in Europe. Huntingdon Life Sciences Ltd. DAS Report No.: R&H 213/971164 (Masterfile Number) ER R95.6 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.3.2/05	Feilden, A.	1998a	To Determine the Magnitude of Residues During the 14 Days Following the Final Application in the Raw Ag. Commodity of Wine Grapes Resulting from Sequential Directed Application of Systhane 24E in Italy and Greece. Huntingdon Life Sciences Ltd. DAS Report No.: RAS 23/974501 (Masterfile Number) ER R101.1 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.3.2/06	Gilbert, J.	1997i	To Determine the Magnitude of Residues of Myclobutanil and the Metabolite RH-9090 During the 14 Days Following the Final Application in the Raw Agricultural Commodity of Wine Grapes Resulting from Sequential Directed Application of Systhane Huntingdon Life Sciences Ltd. DAS Report No.: Derbi 138356; R&H 212 (Masterfile Number) ER R95.5 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.3.2/07	Gilbert, J.	1997j	To Determine the Magnitude of Residues of Myclobutanil and the Metabolite RH-9090 During the 14 Days Following the Final Application in the Raw Agricultural Commodity of Wine Grapes Resulting from Sequential Directed Application of Systhane Huntingdon Life Sciences Ltd DAS Report No.: Derbi 138357; R&H 211 (Masterfile Number) ER R96.1 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.3.2/08	Oxspring, S.	2005c	To Determine the Magnitude of Myclobutanil Residues at Harvest and at Intervals in the Raw Agricultural Commodity Grapes Resulting from Sequential Overall Applications of GF-1317, in Northern France, 2004 Agrisearch UK Ltd DAS Report No.: GHE-P-10966 (Masterfile Number) ER R 106.11 GLP/GEP (Y/N): Y Published (Y/N): N

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not
IIA 6.3.2/09	Oxspring, S.	2005d	To Determine the Magnitude of Myclobutanil Residues at Harvest and at Intervals in the Raw Agricultural Commodity Table Grapes Resulting from Sequential Overall Applications of GF-1317, in Italy and Spain, 2004 Agrisearch UK Ltd DAS Report No.: GHE-P-10965 (Masterfile Number) ER R 106.12 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.4/01	Desai, R., Garstka, T., Cui, Y.	1998	Systhane (myclobutanil) Cow Feeding Study : Magnitude of Residue in Lactating Dairy Cows. [redacted] DAS Report No.: 34-97-31 (Masterfile Number) ER 52.3 GLP/GEP (Y/N): Y Published (Y/N): N
~~IIA 6.5.1~~	~~Betteley, J.~~	~~1994~~	~~RH-3866 Abiotic Degradation : Hydrolysis as a Function of pH.~~ ~~Huntingdon Research Centre Ltd.~~ ~~DAS Report No.: TR 34-94-108~~ ~~(Masterfile Number) ER 43.1~~ ~~GLP/GEP (Y/N): Y~~ ~~Published (Y/N): N~~
IIA 6.5.2.1/01	Distler, B.	1993b	Myclobutanil Apple Residue Studies 1986. Dr Specht Laboratories. DAS Report No.: DEU86F21211 to DEU86F21241 (Masterfile Number) ER R75.14 GLP/GEP (Y/N): N Published (Y/N): N
IIA 6.5.2.1/02	Oxspring, S.	2005e	To Determine the Magnitude of Myclobutanil Residues at Harvest and at Intervals in the Raw Agricultural Commodity Apples and Processed Fractions Resulting from Sequential Overall Applications of GF-1317, in Northern France, 2004 Agrisearch UK Ltd DAS Report No.: GHE-P-10967 (Masterfile Number) ER R 106.9 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.5.2.2/01	Feilden, A.	1998b	To Determine the Magnitude of Residues During the 28 Days Following the Final Application in the Raw Agricultural and Processed Commodity of Wine Grapes Resulting from Sequential Directed Application of Systhane 20EW in Northern France.

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not
			Huntingdon Life Sciences Ltd. DAS Report No.: TR-34-98-43; RAS 18/980226 (Masterfile Number) ER R101.2 GLP/GEP (Y/N): Y Published (Y/N): N
IIA 6.5.2.2/02	Gilbert, J.	1997k	To Determine the Magnitude of Residues of myclobutanil & RH-9090 During the 28 days Following the Final Application in the Raw and Processed Ag. Commodity of Grapes Resulting from Sequential Directed Application of Systhane 20EW in Germany. Huntingdon Life Sciences Ltd. DAS Report No.: R&H 203/971083 (Masterfile Number) ER R95.4 GLP/GEP (Y/N): Y Published (Y/N): N

Open point 3.6: *Please provide information on the radioactive purity and the specific activity of the test substance*

Metabolism, distribution and expression of residues of myclobutanil in plants (Annex IIA 6.1)

-Metabolism of ^{14}-C RH-3866 in Field Treated Grapes – Report TR 310-84-30 (Nelson S.S., 1984b)

Test substances : (Phenyl ring –^{14}C)-myclobutanil and (Triazole ring –^{14}C)-myclobutanil.
*(Phenyl ring –^{14}C)-myclobutanil :
Radiochemical purity : 99 %
Specific activity : 10.28 mCi/g
*(Triazole ring –^{14}C)-myclobutanil :
Radiochemical purity : 99 %
Specific activity : 10.98 mCi/g

-The Metabolism of RH-3866 in Apples (Nelson S.S., Streelman D.R.; 1984c)

Test substances : (Phenyl ring –^{14}C)-myclobutanil and (Triazole ring –^{14}C)-myclobutanil.
*(Phenyl ring –^{14}C)-myclobutanil :
Radiochemical purity : 99 %
Specific activity : 1.99 mCi/g
*(Triazole ring –^{14}C)-myclobutanil :
Radiochemical purity : 99 %
Specific activity : 1.97 mCi/g

-The Metabolism of RH-3866 in Wheat (Streelman D.R., 1984)

Test substances : (Phenyl ring –^{14}C)-myclobutanil and (Triazole ring –^{14}C)-myclobutanil.
*(Phenyl ring –^{14}C)-myclobutanil :
Radiochemical purity : 99 %
Specific activity : 10.28 mCi/g

*(Triazole ring –^{14}C)-myclobutanil :
Radiochemical purity : 99 %
Specific activity : 2.00 mCi/g

Metabolism, distribution and expression of residues of myclobutanil in livestock (Annex IIA 6.2)

Metabolism, distribution and expression of residues of myclobutanil in Lactating cows

-^{14}-C RH-3866 Feeding Study in Cows (Jacobson A.H., 1986a)
-^{14}-C-RH-3866 Dairy Cow Residue Metabolism and Feeding Study (Nelson S.S., 1984)

Test substances : A mixture of ^{14}-C-(phenyl ring)-RH-3866; ^{14}-C-(triazole ring)-RH-9090 and ^{14}-C-(triazole ring)-RH-9089 at a ratio of 32:58:10, w/w/w, respectively.
*^{14}-C-(phenyl ring)-RH-3866
Radiochemical purity : >98%
Specific activity : 10.28 mCi/g
*^{14}-C-(triazole ring)-RH-9090
Radiochemical purity : >98%
Specific activity : 10.20 mCi/g
^{14}-C-(triazole ring)-RH-9089
Radiochemical purity : >98%
Specific activity : 9.78 mCi/g

-Characterization and Identification of Metabolites in Cows fed a ^{14}-C Mixture of RH-3866/RH-9090/RH-9089 (Jacobson A.H., 1986b)

Test substances : Mixture of ^{14}-C-(phenyl ring)-RH-3866; ^{14}-C-(triazole ring)-RH-9090 and ^{14}-C-(triazole ring)-RH-9089, a mixture at a ratio of 32:58:10, w/w/w, respectively.
*^{14}-C-(phenyl ring)-RH-3866
Radiochemical purity :>98 %
Specific activity : 10.28 dpm/µg
*^{14}-C-(triazole ring)-RH-9090
Radiochemical purity :>98 %
Specific activity : 10.20 dpm/µg
*^{14}-C-(triazole ring)-RH-9089
Radiochemical purity :>99 %
Specific activity : 9.78 dpm/µg

Metabolism, distribution and expression of residues of myclobutanil in laying hens

-Technical Report N° 31H-86-16 : ^{14}C RH-3866 Feeding Study in Poultry (Jacobson A., 1986c)

Test substances :
-Mixture of (^{14}C-phenyl-ring) RH-3866/(^{14}C-triazole-ring) RH-9090/(^{14}C-triazole-ring) RH-9089; nominal ratio of (45:45:10, w/w/w) for the groups of hens 2 through 5;
-^{14}-C labelled mixture of RH-9090/RH-9089- (^{14}C-triazole-ring); nominal ratio of (82:18, w/w) for the group of hens 7;
-^{14}-C labelled RH-3866 for the group of hens 6.

*^{14}-C-(phenyl ring)-RH-3866
Radiochemical purity :95 %
Specific activity : 10.98 mCi/g
*^{14}-C-(triazole ring)-RH-9090
Radiochemical purity :95.9 %
Specific activity : 11.40 mCi/g
*^{14}-C-(triazole ring)-RH-9089

Radiochemical purity :99 %
Specific activity : 9.78 mCi/g

- Technical Report N° 31H-86-17 : Disposition and Metabolism of RH-3866 and Metabolites in Laying Hens (Martin J.J., 1986)

Test substances : ^{14}C-RH-3866 (hen group 6) and ^{14}C-RH-9090/RH-9089 (ratio of 82:18, w/w) (hen group 7).
*^{14}-C-(phenyl ring)-RH-3866
Radiochemical purity : 95 %
Specific activity : 10.98 mCi/g
*^{14}-C-(triazole ring)-RH-9090
Radiochemical purity :95.9 %
Specific activity : 11.40 mCi/g
*^{14}-C-(triazole ring)-RH-9089
Radiochemical purity :99 %
Specific activity : 9.78 mCi/g

Open point 3.7 :

-Metabolism of ^{14}-C RH-3866 in Field Treated Grapes – Report TR 310-84-30 (Nelson S.S., 1984b)

-*Extraction procedure for whole fruit* : None.
The harvested grapes were processed into pomace and juice and the residue levels as well as the metabolites identification in whole grapes were calculated/investigated from the residues in processed juice and pomace (87.5 % of the residue were concentrated in the pomace fraction).
Therefore, no extraction procedure was used for the whole grapes and no residual radioactive residues were determined.
Total identified metabolites : 79 % TRR (0.191 ppm)-Triazol labelling to 82 % TRR (0.26 ppm)-phenyl labelling.

-*Extraction procedure for juice* :
Juice was made at pH 7 with sodium bicarbonate followed by partitioning against chloroform and 1-butanol to provide the organosoluble and water soluble partitioned phases.
The chloroform fraction contained most of the radioactivity that was investigated by TLC analysis.
The 1-butanol and aqueous fractions were difficult to examine because of the nature of the matrix.
No residual radioactive fraction resulted from this partitioning procedure.
The 1-butanol and aqueous fractions were difficult to examine because of the plant matrix.
The aqueous fraction was further submitted to enzymatic hydrolysis (α- and β-glucosidases) followed by partitioning against chloroform to identify mainly the following metabolite : RH-9090 glucoside in the juice. The remaining activity in the aqueous fraction was not characterized.
Total identified metabolites : 60 % TRR (0.02 ppm)-Triazol labelling to 84 % TRR (0.035 ppm)-phenyl labelling.

-*Extraction procedure for* ***wet pomace*** :
Wet pomace was extracted with methanol followed by partitioning against hexane. The aqueous methanol was then concentrated and partitioned against chloroform.
TLC analysis for isolation/characterization of the metabolites was performed on the chloroform extracts and on the aqueous fractions of wet pomace.
Total identified metabolites : 82 % TRR (0.746 ppm)-Triazol labelling to 82 % TRR (0.793 ppm)-phenyl labelling.

-*Extraction procedure for foliage*:
The total radioactive residues were not given for grape foliage for both the 2 labelling forms.
Grape foliage was extracted with methanol followed by successive partitioning against hexane, chloroform and 1-butanol.
Total identified metabolites : 78-80 % of the TRR.

According to the EU guidance doc. 7028/VI/95, if the non extractable residues are less than 0.05 mg/kg or 25 % of the TRR and a significant proportion of the total residues has been identified, then no further work is required. Therefore, based on the results in the table B.7.1.1-2 in the DAR, the metabolism of myclobutanil in grapes has been sufficiently investigated.

Open points 3.9/3.11 :

- Technical Report N° 31H-86-17 : Disposition and Metabolism of RH-3866 and Metabolites in Laying Hens (Martin J.J., 1986)

1)Extraction procedure : (reference is made to Table B.7.2.2-3 in the DAR)

**Whole eggs* :
Lyophilised eggs were extracted with Ethyl Acetate several times and the combined EtoAc fractions were concentrated and then extracted with methanol with further concentration.
-The EtoAc residues were fractionated on a Florisil column. The radioactivity was eluted with methanol. Radioactive fractions were combined and concentrated.
-The methanol residues were applied to a silica Sep-Pak cartridge, washed with different solvents. Fractionation of the residues in methanol was performed by preparative TLC.
The EtoAc and methanol residues were identified by TLC analysis by co-chromatography with reference compounds (RH-3866, RH-9090, RH-9089, RH-9090-sulfate, RH-294 and the hydroxy-lactone).

**Breast/thigh muscle* :
Samples of muscles were extracted with EtoAc several times and the extracts were combined.
The tissues were then extracted with methanol followed by a Soxhlet extraction with methanol.
-The EtoAc fractions were evaporated to dryness and applied to a Florisil Column yielding a non polar fraction and a methanol fraction.
-The methanol extract was concentrated to dryness and re-dissolved in methanol (soxhlet extraction phase).
-The soxhlet extract was applied to a C18 SPE cartridge and the radioactivity was eluted with methanol followed by analysis by HPLC.
Each fraction was concentrated to dryness, re-dissolved in methanol and analysed by HPLC by chromatographic comparison (retention times) with reference standards.

**Fat* :
Samples of fat from 2 distinct groups of hens –groups 6 (^{14}C-phenyl label-parent) and 7 (^{14}C-Triazolyl label-RH9090/RH-9089) (see test substances in the DAR –B.7.2.2) were extracted respectively with n-hexane and n-heptane both followed by partitioning against methanol.
The methanol partitioned phases were concentrated on roto evaporator and dissolved in toluene.
Individual samples were applied to a Florisil Sep-Pack and eluted with methanol /Toluene and analysed by TLC with chromatographic comparison with reference standards (RH-3866, RH-9090, RH-9089, RH-9090-sulfate and RH-294).

**Liver and kidney* :
Samples were extracted with methanol followed by methanol soxhlet extraction. Water was added to the combined methanol extracts and the methanol was removed by rotary evaporator. The resulting aqueous solution was extracted successively with n-hexane and Ethyl Acetate.
The aqueous liver extract was further extracted with n-butanol.
The aqueous kidney extract was further fractionated by SPE yielding an aqueous and a methanolic fraction.
The hexane and EtoAc fractions were fractionated and analysed by TLC and HPLC analysis.
The n-butanol extract of the liver and the methanol eluate from the SPE analysis of the kidney were analysed by HPLC.

Table B.7.2.2-3 : Material balance and metabolites distribution of the residues of myclobutanil in eggs and edible tissues of the laying hens after oral administration of ^{14}C-RH-3866 (group of hens 6) and ^{14}C-RH-9090/RH-9089 (ratio of 82:18, w/w) (group of hens 7) at a nominal dietary intake of 110 mg/kg diet - Residues expressed in percent of the total radioactive residues and in *(mg myclobutanil equiv./kg)*.

Labelling forms	**^{14}C-RH-3866 (Hen group 6)**						**^{14}C-RH-9090/RH-9089 (ratio of 82:18, w/w) (Hen group 7)**					
Tissues	**Whole eggs[1]**	**Fat**	**Breast muscle**	**Thigh muscle**	**Liver**	**Kidney**	**Whole eggs[1]**	**Fat**	**Breast muscle**	**Thigh muscle**	**Liver**	**Kidney**
Total radioactive residues in % of TRR - *(mg myclobutanil equivalent/kg)*												
	100 *(1.006-1.746)*	100 *(0.017)*	100 *(0.060)*	100 *(0.056)*	100 *(0.52)*	100 *(0.32)*	100 *(1.349-1.969)*	100 *(0.010)*	100 *(0.077)*	100 *(0.065)*	100 *(0.31)*	100 *(0.16)*
Extractability of radioactive residues in % of TRR - *(mg myclobutanil equivalent/kg)*												
Ethyl acetate extraction phase [1]	40 *(0.698)*	Not given	54 *(0.0324)*	49 *(0.0294)*			28 *(0.551)*	Not given	48 *(0.0369)*	42 *(0.0273)*		
Methanol extraction phase [2]	29 *(0.506)*		29 *(0.0174)*	14 *(0.0078)*	?	?	49 *(0.964)*		24 *(0.0184)*	19 *(0.0123)*	?	?
Soxhlet methanol extraction phase [3]			14 *(0.0084)*	20 *(0.0112)*	?	?			14 *(0.0107)*	11 *(0.0071)*	?	?
Hexane partitioned phase [4]					35.01 *(0.1821)*	7.68 *(0.0246)*					43.74 *(0.1356)*	7.62 *(0.0122)*
Ethyl acetate partitioned phase [5]					39.3 *(0.2044)*	25.53 *(0.0817)*					42.77 *(0.1326)*	30.81 *(0.0493)*
n-butanol partitioned phase [6]					5.25 *(0.0273)*	Not performed					11.19 *(0.0347)*	Not performed
Methanol eluate from C18 separation [7]					Not performed	9.4 *(0.0301)*					Not performed	18.12 *(0.0290)*
Water soluble phase [8]					4.26 *(0.0222)*	0.718 *(0.0023)*					5.0 *(0.0155)*	1.875 *(0.0030)*
Elucidation of radioactive residues in % of TRR and - *(mg myclobutanil equivalent/kg)*												
RH-3866 parent	ND	85.0 *(?)*	7.9* *(0.0025)*	3.3* *(0.00097)*	43.0 *(0.087)*	30.0 *(0.0245)*	ND	ND	ND	ND	ND	ND

Labelling forms	**^{14}C-RH-3866 (Hen group 6)**						**^{14}C-RH-9090/RH-9089 (ratio of 82:18, w/w) (Hen group 7)**					
Tissues	**Whole eggs[(1)]**	**Fat**	**Breast muscle**	**Thigh muscle**	**Liver**	**Kidney**	**Whole eggs[(1)]**	**Fat**	**Breast muscle**	**Thigh muscle**	**Liver**	**Kidney**
Alcohol RH-9090	55*/47** *(0.383*/ 0.237**)*	ND	ND	ND	ND	ND	67*/58** *(0.369*/ 0.559**)*	23.0 *(?)*	ND	ND	ND	ND
Alcohol RH-9090 sulfate	8*/5** *(0.055*/ 0.025**)*	ND	7.9** *(0.002)*	8.4** *(0.0015)*	20.0 *(0.04)*	ND	2** *(0.019)*	ND	9.7** *(0.0028)*	18.2** *(0.0035)*	ND	ND
Ketone RH-9089	21*/7** *(0.146*/ 0.035**)*	ND	85.0*/81.4** *(0.0275*/ 0.021**)*	88.4*/70.8** *(0.025*/ 0.0134**)*	ND	ND	10** (0.096)	ND	93.2*/82.0** *(0.034*/ 0.023**)*	94.8*/65.3** *(0.0258*/ 0.0126**)*	ND	ND
Diol RH-294	15** *(0.075)*	ND	10.8** *(0.0027)*	7.9*/18.6** *(0.0023*/ 0.0035**)*	ND	7.0 *(0.0057)*	14** *(0.134)*	ND	6.4** *(0.0018)*	3.9*/9.7** *(0.001*/ 0.00188**)*	14 *(0.0185)*	10 *(0.00493)*
4-hydroxy-3-Lactone metabolite	17*/19** *(0.118*/ 0.096**)*	12.0 *(?)*	ND	ND	ND	ND	14*/16** *(0.077*/ 0.154**)*	27.0 *(?)*	ND	ND	ND	
Undissociated 4-hydroxy-3-Lactone /RH-9090/RH-9089	ND	ND	ND	ND	35.0 *(0.071)*	39.0 *(0.031)*	ND	ND	ND	ND	85 *(0.112)*	84 *(0.0414)*
Unknown metabolites	8** *(0.04)*	3.0 *(?)*	ND	ND			19* *(0.104)*	51.0 *(?)*	2.0** *(0.00058)*	6.8** *(0.00131)*	ND	
Total identified metabolites	101*/93** *(0.702*/ 0.468**)*		92.9*/100.1** *(0.03*/ 0.0257**)*	99.6*/97.8** *(0.0287*/ 0.0184**)*	98.0 *(0.198)*	76.0 *(0.0612)*	81*/100** *(0.446*/ 0.962**)*		93.2*/98.1** *(0.034*/ 0.0276**)*	98.7*/93.2** *(0.0268*/ 0.0179**)*	99.0 *(0.131)*	94.0 *(0.0463)*
Residual radioactive residues in % of TRR - *(mg myclobutanil equivalent/kg)*												
	31.0 *(0.541)*	Not given	21 *(0.0126)*	32 *(0.0179)*	16.18 *(0.084)*	56.68 *(0.1387)*	23.0 *(0.452)*	Not given	20 *(0.0154)*	18 *(0.0117)*	Not given	Not given
Accountability : Partitioned phases + residual radioactive residues in % of TRR - *(mg myclobutanil equivalent/kg)*												
	100.0 *(1.745)*		118 *(0.0708)*	115 *(0.0644)*	100.0 *(0.52)*	100.0 *(0.32)*	100.0 *(-)*		106 *(0.081)*	90 *(0.058)*		

Labelling forms	**^{14}C-RH-3866 (Hen group 6)**						**^{14}C-RH-9090/RH-9089 (ratio of 82:18, w/w) (Hen group 7)**					
Tissues	**Whole eggs(1)**	**Fat**	**Breast muscle**	**Thigh muscle**	**Liver**	**Kidney**	**Whole eggs(1)**	**Fat**	**Breast muscle**	**Thigh muscle**	**Liver**	**Kidney**
Np : value not provided. Nd : Not radiodetected. *: Metabolites characterized/identified in the ethyl acetate extraction phase ** : Metabolites characterized/identified in the methanol /soxhlet methanol extraction phases (1) : The total radioactive residues in lyophilised whole eggs were determined on sample days 4 and 7 for both the hen groups 6 and 7. *Remarks* : Identification of the metabolites was performed on the following fractions indicated as follows: -(1)/(2) for whole eggs, -(5) for liver and kidney, -(1)/(2)/(3) for muscles *Liver and kidney* : The TRR values in the methanol extraction phase and the Soxhlet methanol extraction phase were not provided. The counts (dpm) of the methanol extract were provided but it was not possible to calculate the radioactivity recovered due to the fact that the amount of tissue extracted was not presented in the raw data. No indication of the Soxhlet methanol extraction counts was provided. *Fat* : The determination of the TRR in the different extraction phases and for the metabolites recovered was not possible in fat as no data was provided on the extractability pattern.												

Table B.7.2.2-4 Identification of the metabolites in the hen matrices from hen goups 6 and 7 - Summary

Labelling forms	**^{14}C-RH-3866 (Hen group 6)**						**^{14}C-RH-9090/RH-9089 (ratio of 82:18, w/w) (Hen group 7)**					
Tissues	**Whole eggs**	**Fat[1]**	**Breast muscle**	**Thigh muscle**	**Liver**	**Kidney**	**Whole eggs**	**Fat[1]**	**Breast muscle**	**Thigh muscle**	**Liver**	**Kidney**
Total radioactive residues in % of TRR - *(mg myclobutanil equivalent/kg)*												
	100 *(1.746)*	100 *(0.017)*	100 *(0.060)*	100 *(0.056)*	100 *(0.52)*	100 *(0.32)*	100 *(1.969)*	100 *(0.010)*	100 *(0.077)*	100 *(0.065)*	100 *(0.31)*	100 *(0.16)*
Elucidation of radioactive residues in % of TRR												
RH-3866 parent	nd	detected	4.26	1.61	16.89	7.65	nd	nd	nd	nd	nd	nd
Alcohol RH-9090	35.63		Nd	Nd	Nd	Nd	47.85	detected	Nd	Nd	nd	nd
Alcohol RH-9090 sulfate	4.65		3.39	2.85	7.86	Nd	0.98		3.68	5.46	nd	nd
Ketone RH-9089	10.07		80.9	67.38	Nd	Nd	4.9		75.86	59.4	nd	nd
Diol RH-294	4.35		4.64	10.19	Nd	1.78	6.86		2.43	4.54	5.98	3.081
4-hydroxy-3-Lactone metabolite	12.31	detected	Nd	Nd	Nd	Nd	11.76	detected	Nd	Nd	nd	
Undissociated 4-hydroxy-3-Lactone /RH-9090/RH-9089	Nd		Nd	Nd	13.75	9.95	Nd		Nd	Nd	36.35	25.88
Unknown metabolites	2.32		-	-	-	-	5.32		0.76	2.04	-	-
Total identified metabolites	69.33		93.19	82.03	38.5	19.38	72.35		81.97	69.4	42.33	28.96
(1) :The determination of the TRR for the different metabolites recovered was not possible in fat as no data was provided on the extractability pattern.												

2) In Table B.7.2.2-3, a fraction corresponding to undissociated lactone/RH-9090/RH-9089 was elucidated in poultry liver and kidney.
The Ethyl acetate extract was resolved by HPLC into fractions with retention times matching with the following reference compounds parent, RH-0294 (diol), RH-9090, RH-9089 and the hydroxy-lactone.
The highest percent of radioactive residues were found in the RH-9090, RH-9089 and the 4-hydroxy-3-lactone area for liver and kidney (36 % of TRR and 26 % of TRR, respectively) for the hen group 7 while the group 6 liver and kidney matrices contained approximately equal amounts of radioactive residues in the RH-9090/RH-9089/4-hydroxy-3-lactone area (13 % and 10% of the TRR, respectively).

Open point 3.10:

-Characterization and Identification of metabolites in Cows fed a ^{14}C-Mixture of RH-3866/RH-9090/RH-9089 (Jacobson A.H. 1986b)

1) RMS agrees that only the parent compound labelled respectively on the phenyl ring and the triazole ring should be used as the test substances in the experimental design according to the EU guideline.
The notifier did not provide any rationale for this study design.
The metabolic pattern in cow tissues consisted of compounds structurally related to the parent Myclobutanil.
It is true that, as observed in wheat, it cannot be excluded that the cleavage of the phenethyl triazole linkage may occur in livestock and that the resulting triazole derivative metabolites can be generated but not detected since only the phenyl ring labelling form of Myclobutanil was fed to the lactating cows.
However, the "mixture" of test substances still included the alcohol RH-9090 and the ketone RH-9089 labelled on the triazole ring, respectively.
The notifier presented the following rationale : a mixture containing RH-3866/RH-9090/RH-9089 was fed to cows and laying hens to reflect the potential field exposure of these species to treated crops.

2) The log $P_{o/w}$ value of 3.17 for Myclobutanil was considered as acceptable. The partition coefficient n-octanol/water for the other metabolites was not established by the notifier.

3) Recalculation of livestock dietary burden was made under open point 3.16.

4) For each matrix, the extraction pathway and the subsequent partitioning in solvent systems are described as follows :

*Milk :
The distribution of radioactivity in the different milk fractions showed that the milk solids (36.6%) and the soluble whey (58.5%) fractions contained over 95 % of the radioactivity recovered. The rest of radioactivity concerned fat, proteins and lactose.
Milk samples were centrifuged to remove fat. The fat pad was removed and the supernatant was decanted. The pellets were washed with water , centrifuged and the resulting fat and supernatant were added to the previous samples. Aqueous samples were combined and the remaining milk solids were dried.
-*The milk solids* were extracted with water. The supernatants were combined and concentrated. The **water insoluble residues were Soxhlet extracted with methanol.**
The water extractable milk solids were analysed by HPLC and the observable radioactivity was in the RH-9090/9089 and RH-294 regions.
The Soxhlet methanol extracted phase was also characterized by TLC and HPLC analysis and revealed the presence of RH-294 and RH-9089/9090.
Acetonitrile was added to the skimmed milk to precipitate the proteins. Lactose was precipitated from the aqueous solution using 2-propanol and was separated from the whey-soluble material by filtration.
-*The whey soluble fraction* was washed with water and the radioactivity was eluted with methanol followed by partitioning against hexane. **The methanol/water fraction was analysed by HPLC.**

*Liver and kidney : were extracted with Ethyl Acetate. The resulting fractions were concentrated and centrifuged to remove fatty residues. The supernatant was dissolved in petroleum ether and extracted several times with water. The combined water fractions were applied to a C18 SPE cartridge and eluted with ethanol.
The Ethyl Acetate fraction followed by C18 clean-up and elution with ethanol constituted the only reported fraction used for identification.

This fraction was applied to a preparative-TLC to fractionate the radioactivity followed by elution with chloroform : methanol and the individual radioactive zones were submitted to GC-MS analysis for further structural information.

The bound residues remaining after Ethyl Acetate extraction were further extracted with ethanol and the ethanol extracts were dried and Soxhlet extracted with methanol.

Lipids were extracted from the ethanol and methanol/Soxhlet extraction phases by dissolving the residues into chloroform/methanol/water.

The methanol/water samples were then diluted with water and chloroform. The chloroform fraction was evaporated and dissolved in water. The aqueous fraction was applied to a C18 SPE column and radioactivity eluted with methanol which was then analysed.

5) The concentrations of Myclobutanil (mg myclobutanil equiv./kg) in urine, feces, milk and tissues were not provided in this study (Table B.7.2.1-2) but were extracted from the study : "^{14}C-RH-3866 Dairy Cows – Residue Metabolism and Feeding Study (Nelson S.S., 1984) as the experimental design is similar to that of the study from Jacobson A.H., 1986b : *"Characterization and Identification of Metabolites in Cows fed a ^{14}C-Mixture of RH-3866/RH-9090/RH-9089"*.

Table B.7.2.1-2 (DAR) : Magnitude of the myclobutanil residue levels in lactating cows excreta, milk and tissues following daily oral administration of ^{14}-C-labelled mixture of RH-3866, RH-9090 and RH-9089 at the following actual feeding levels : 0.915, 3.05, 9.15 and 30.5 mg/kg in diet for 10 consecutive days (Residues expressed as mg Myclobutanil equivalents/kg).

Dose level (mg/kg in diet)/samples	**0.3 X Treatment group (0.915 mg/kg)**	**1 X Treatment group (3.05 mg/kg)**	**3 X Treatment group (9.15 mg/kg)**	**10 X Treatment group (30.5 mg/kg)**
Urine	0.39	1.2	3.5	10
Feces	0.25	0.87	1.6	5.9
Milk	0.008	~~0.029~~ 0.019	~~0.095~~ 0.065	~~0.248~~ 0.16
Muscle type 1	<0.02	<0.02	<0.02	0.024
Muscle type 2	<0.02	<0.02	<0.02	0.038
Muscle type 3	<0.02	<0.02	<0.02	0.022
Fat perirenal	<0.02	<0.02	<0.02	0.022
Fat omental	<0.02	<0.02	<0.02	0.022
Fat mediastinal	<0.02	<0.02	<0.02	0.022
Kidney	<0.02	<0.02	0.050	0.15
Blood	<0.02	<0.02	0.030	0.059
Liver	0.045	0.11	0.30	0.82
Gall Bladder contents	0.22	0.40	1.7	2.7

Remarks :
-Results are based on an average of the 2 animals in each dose group.
-Urine, feaces and milk samples are from day 10; tissue samples are taken from day 11.
Limit of quantification of the analytical method for tissues : 0.02 mg/kg.
Limit of quantification of the analytical method for milk : 0.005 mg/kg.
-Muscle type 1 : longissimus dorsi;
-Muscle type 2: semimembranosus;
-Muscle type 3 : triceps.

Milk Sampling day	**Day 3**	**Day 6**	**Day 10**
Total radioactive residues % TRR and mg Myclobutanil equiv./kg)			
Milk solids	45.3 % (*0.072 mg/kg*)	38.1 % (*0.06 mg/kg*)	26.3 % (*0.042 mg/kg*)
Whey soluble fraction	50.2 % (*0.08 mg/kg*)	56.6 % (*0.09 mg/kg*)	68.8 % (*0.11 mg/kg*)

Table B.7.2.1-4 (DAR): Balance, characterization and identification of radioactive residues in milk and tissues of cows treated at a dose rate of 30.5 ppm with a (^{14}C)-mixture of RH-3866, RH-9090 and RH-9089 (32:58:10, w/w/w) for 10 consecutive days - Residues expressed as percentage of total radioactive residues and in *(mg Myclobutanil equiv./kg)*.

Tissues	**Milk solids fraction** [1]	**Whey soluble fraction**[1]	**Liver**	**Kidney**
Total radioactive residues (mg myclobutanil equiv./kg)	0.058 (0.042-0.072)	0.093 (0.08-0.11)	0.82	0.15
Extractability of radioactive residues - % of TRR and *(mg myclobutanil equivalent/kg)*				
Water extraction phase	(46.8-16.8) [1] *(0.027-0.009)*			
Ethyl acetate extraction phase			38.8 *(0.318)*	23.3 *(0.034)*
Ethanol extraction phase			30.5 *(0.25)*	39.7 *(0.059)*
Methanol Soxhlet extraction phase	(21.2-27.4) [1] *(0.012-0.0158)*		21.5 *(0.17)*	14.5 *(0.021)*
Partitioning of extracted residues - % of TRR and *(mg myclobutanil equivalent/kg)*				
Petroleum ether partitioned phase			3.2 *(0.026)*	2.6 *(0.003)*
$CHCL_3$ partitioned phase			29.1 *(0.23)*	16.3 *(0.024)*
Aqueous soluble phase			41.2 *(0.33)*	51.8 *(0.077)*
Elucidation of total radioactive residues - % of TRR and *(mg myclobutanil equivalent/kg)*				
4, 5-diol metabolite RH-294		(32.75-27.22) [1] *(0.03-0.025)*	~~6.7~~ *(0.054)* 2.04 *(0.016)*	~~41.4~~ *(0.062)* 16.43 *(0.024)*
Monohydroxyl RH-9090		(21.15-5.47)[1] *(0.019-0.005)*	~~42.5~~ *(0.348)* 12.96 *(0.106)*	~~23.4~~ *(0.035)* 9.28 *(0.013)*
Ketone RH-9089			~~3.8~~ *(0.031)* 1.15 *(0.009)*	~~13.1~~ *(0.019)* 5.20 *(0.0078)*
4-Hydroxy-3-lactone metabolite			~~46.4~~ *(0.38)* 14.1 *(0.115)*	~~22.1~~ *(0.033* 8.77 *(0.013))*
Polar unknown metabolites[2]		(45.6-67.32) [1] *(0.042-0.062)*		
Total identified metabolites		(53.9-32.69) [1] *(0.05-0.03)*	~~99.4~~ *(0.815)* 30.25 *(0.248)*	~~100.0~~ *(0.15)* 39.68 *(0.059)*
Residual radioactive residues - % of TRR and *(mg myclobutanil equivalent/kg)*				
	(32.0-55.8) [1] *(0.0185-0.032)*		26.9 *(0.22)*	21.0 *(0.0315)*
Accountability (extracted phases + RRR)				
	100.0		117.7	98.5

Tissues	Milk solids fraction (1)	Whey soluble fraction(1)	Liver	Kidney
(1): Metabolites were identified in the soluble whey fraction of milk from day 3, day 6 and day 10 of the study. *Remarks* : -No identification of metabolites was attempted on the milk solid fractions. -Only milk, tissues and urine from cows treated at the 30 ppm dose level were analysed for metabolite characterization. -TRR in milk : 0.16 mg myclobutanil equiv./kg. -No metabolite characterization/identification in muscle and fat was attempted due to the very low level of total residues (at or below the Limit of Quantification). Distribution of radioactivity in milk : Milk solids (36 % TRR), whey solubles (58.5 % TRR), Fat (1.8 % TRR), Proteins (1.7 % TRR), lactose (1.0 % TRR). Note : (2) : 3 polar metabolites co-chromatographed with bands isolated from urine and characterized as conjugates of RH-9090 and RH-9089.				

6) No metabolite characterization/identification in muscle/fat was attempted due to the very low level of total residue (at or below the Limit of Quantification-0.02 mg/kg).

7) RMS agrees that the metabolite RH-294 is a diol and not a carboxylic acid.

Open point 3.16 :

A) *Definition of the residues in plants matrices (apples and grapes)*:
Enforcement purposes : Myclobutanil.
Risk assessment : Myclobutanil and RH-9090 expressed as myclobutanil.

B) *Analytical methods* :
As it is stated in the DAR under chapter B.7.6, different analytical methods were developed and considered as sufficiently validated for the determination of the residues of Myclobutanil and its alcohol metabolite RH-9090 in the residue trials.

C) *Processing data* :
The residues levels of Myclobutanil and its alcohol metabolite RH-9090 in apples and grapes RACs and in their processed products were reported in Table B.7.7.2-1 (DAR) here below :

Table B.7.7.2-1 : Determination of the residue transfer factor for myclobutanil and metabolite RH-9090 in the different processed fractions of apples and grapes obtained from the residue trials characterized as here above (Residues expressed as mg myclobutanil equivalents/kg).

Residue trial references	RAC/Processed commodities	Myclobutanil/RH-9090 residues in whole fruit	Myclobutanil /RH-9090 residues in processed fractions	Transfer factors (RAC/processed fraction) for myclobutanil	% of transference
Apples					
Trial N°DEU86F21221	**Unwashed whole fruit**	0.145/<0.01	na		
	Wet pomace	na	0.080/<0.01	*0.55*	-
	Juice	na	0.024/<0.01	*0.165*	-
Trial N°DEU86F21241	**Unwashed whole fruit**	0.348/<0.01	na		
	Wet pomace	na	0.225/<0.01	*0.646*	-
	Juice	na	0.037/<0.01	*0.106*	-
Trial AF/8164/DE/4 GHE-P-10967 (Treatment at the critical GAP rate)	**Unwashed whole fruit**	0.08/<0.01			
	Washed fruit		0.08/<0.01	*1.0*	*100.33*
	Raw juice		0.01/<0.01	*0.125*	*7.9*
	Wet pomace		0.23/0.01	*2.87*	*100.1*

Residue trial references	RAC/Processed commodities	Myclobutanil/RH-9090 residues in whole fruit	Myclobutanil /RH-9090 residues in processed fractions	Transfer factors (RAC/processed fraction) for myclobutanil	% of transference
		Apples			
	Dried pomace		0.99/0.06	*12.37*	*71.96*
	Juice		<0.01/<0.01	*0.125*	*7.9*
	Cooked apple		0.04/<0.01	*0.5*	*50.0*
	Puree		0.02/<0.01	*0.25*	*12.0*
Trial AF/8164/DE/4 GHE-P-10967 (Treatment rate is 5 fold the critical GAP rate)	**Unwashed whole fruit**	0.51/0.05			
	Washed fruit		0.52/0.03	*1.019*	*102.18*
	Raw juice		0.06/0.02	*0.117*	*6.5*
	Wet pomace		1.57/0.05	*3.07*	*97.49*
	Dried pomace		6.0/0.25	*11.76*	*57.9*
	Juice		0.06/0.02	*0.117*	*6.5*
	Cooked apple		0.28/0.03	*0.54*	*53.91*
	Puree		0.13/0.02	*0.25*	*12.14*
		Grapes			
Trial N°RH/203/2/G	**Unwashed whole white grapes**	0.41/0.02	na		
	Juice	na	0.09/<0.01	*0.219*	*15.33*
	Young wine	na	0.06/<0.01	*0.146*	*2.90*
	Mature wine	na	0.07/<0.01	*0.170*	
Trial N°RH/203/3/G	**Unwashed whole red grapes**	0.34/0.015	na		
	Juice	na	0.07/<0.01	*0.205*	
	Young wine	na	0.04/<0.01	*0.117*	
	Mature wine	na	0.04/<0.01	*0.117*	
Trial N°RAS/18/4/F	**Unwashed whole red grapes**	0.51/0.03	na		
	Juice	na	0.08/0.01	*0.156*	*10.16*
	Young wine	na	0.04/0.01	*0.078*	*3.08*
	Mature wine	na	0.05/0.02	*0.098*	

Na : not applicable
- : Material balance not available.
RAC : Raw agricultural commodity.
Limit of Quantification for all the processed commodities : 0.01 mg/kg.
Remark :
Grapes : no data were provided on raisins and pomace.

D) *Storage stability data* :
Data were reported in the DAR for both Myclobutanil and its alcohol metabolite RH-9090 in almond hulls and meat, in cucumbers and tomatoes with the conclusion that acceptable storage stability of the parent compound and RH-9090 was observed for up to 36 months (cucumbers, tomatoes) and up to 18 months (almond hulls and meat).
Data were also reported for both Myclobutanil and its alcohol metabolite RH-9090 in muscle, liver and milk and showed acceptable storage stability of the residues of Myclobutanil and RH-9090 for up to 80 days (muscle, liver) and 15 months (milk).

E) *Revised livestock dietary burden calculation based on the new residue definition on apples and grapes for risk assessment (Myclobutanil + RH-9090 expressed as myclobutanil).*

Intake calculations for dairy cattle (maximum daily intake of dry matter : 20 kg for 550 kg body weight).

Material	% of total DM/day	Intake of DM from material (kg/animal/day)	% dry matter in material	Intake of fresh material (kg/animal/day)	Residue in material (mg/kg)	Residue intake (mg/animal/day)	Intake by crop
Apple pomace (wet)	10	2	23	8.69	0.712	6.187	6.187
Mg/animal/day :							**6.187**
Mg/kg bw/day :							**0.0112**
Mg/kg diet :							**0.311**
Highest residue value of myclobutanil and its alcohol metabolite RH-9090 recovered in the residue trials for apple whole fruit : 0.380 + 0.02 ppm Average Transfer factor for apple wet pomace is 1.78 for myclobutanil.							

Intake calculations for beef cattle (maximum daily intake of dry matter : 15 kg for 350 kg body weight).

Material	% of total DM/day	Intake of DM from material (kg/animal/day)	% dry matter in material	Intake of fresh material (kg/animal/day)	Residue in material (mg/kg)	Residue intake (mg/animal/day)	Intake by crop
Apple pomace (wet)	30	4.5	23	19.56	0.712	13.926	13.926
Mg/animal/day :							**13.926**
Mg/kg bw/day :							**0.0397**
Mg/kg diet :							**0.945**
Highest residue value of myclobutanil and its alcohol metabolite RH-9090 recovered in the residue trials for apple whole fruit : 0.380 + 0.02 ppm Average Transfer factor for apple wet pomace is 1.78 for myclobutanil.							

F) *Revised consumer dietary risk assessment based on the new residue definition on apples and grapes for risk assessment (Myclobutanil + RH-9090 expressed as myclobutanil).*

-*Chronic dietary risk assessment* :

Adult consumer

Commodity	Consumption (kg/day/person)	MRL (mg/kg)	Intake (mg/kg)
Apples	0.040	0.5	0.02
Apple juice	0.0038	0.5	0.0019
Table grapes	0.0161	1	0.0161
Wine grapes (wine)	0.0978	1	0.0978
TOTAL			0.1358

Taking into account a person of 60 kg body weight, the TMDI is 0.0022 mg/kg b.w./day. This represents **9.05 % of the ADI** (0.025 mg/kg b.w./day)

German 4-6 years old girl

Commodity	Consumption (kg/day/person)	MRL (mg/kg)	Intake (mg/kg)
Apples, total	0.1949	0.5	0.097
Table grapes, total	0.0205	1	0.0205
TOTAL			0.1271

Taking into account a girl of 16.1 kg body weight, the TMDI of the German 4-6 years old girl is 0.0078 mg/kg b.w./day. This represents **31.57 % of the ADI** (0.025 mg/kg b.w./day)

UK model

		TOTAL INTAKE based on 97.5th percentile									
		ADULT	INFANT	TODDLER	4-6 YEARS	7-10 YEARS	11-14 YEARS	15-18 YEARS	VEGETARIAN	ELDERLY (OWN HOME)	ELDERLY (RESIDENTIAL)
mg/kg bw/day		0,00121	0,00112	0,00214	0,00128	0,00110	0,00057	0,00073	0,00127	0,00086	0,00026
% of ADI		5%	4%	9%	5%	4%	2%	3%	5%	3%	1%

	STMR	P	COMMODITY INTAKES									
Commodity	(mg/kg)		(mg/kg bw/day)									
Apples	0,115		0,00031	0,00097	0,00171	0,00108	0,00086	0,00047	0,00041	0,00038	0,00025	0,00012
Table grapes	0,09		0,00012	0,00015	0,00042	0,00019	0,00023	0,00010	0,00006	0,00018	0,00012	0,00004
Wine grapes	0,09		0,00089	0,00011	0,00008	0,00008	0,00003	0,00009	0,00032	0,00087	0,00060	0,00013
Wine	0,09	###	0,00006	0,00001	0,00001	0,00001	0,00000	0,00001	0,00002	0,00006	0,00004	0,00001

* 0.00000 corresponds to <0.000005 mg/kg bw/day (any value ≥0.000005 is rounded to 0.00001

L/C Low consumption (<0.1 g/day) or low number of consumers (<4)

- *Short-term dietary intake risk assessment* :

UK model

Goto Inputs

Acute Intakes (97.5th percentiles)

			adult				infant				toddler				4-6 year old child				7-10 year old child	
commodity	HR	P	NESTI	%ARfD	NESTI max	%ARfD max	NESTI	%ARfD	NESTI max	%ARfD max	NESTI	%ARfD	NESTI max	%ARfD max	NESTI	%ARfD	NESTI max	%ARfD max	NESTI	%ARfD
Apples	0,40		0,00598	1,9			0,03919	12,6			0,02882	9,3			0,02230	7,2			0,01644	5,3
Wine	0,54	0,1	0,00101	0,3			0,00033	0,1			0,00020	0,1			0,00027	0,1			0,00008	0,0
Wine grapes	0,54		0,01281	4,1			0,00420	1,4			0,00254	0,8			0,00342	1,1			0,00100	0,3
Table grapes	0,54		0,01065	3,4			0,01552	5,0			0,03296	10,6			0,02722	8,8			0,02505	8,1

commodity	HR	P	11-14 year old child NESTI	%ARfD	NESTI max	%ARfD max	15-18 year old child NESTI	%ARfD	NESTI max	%ARfD max	vegetarian NESTI	%ARfD	NESTI max	%ARfD max	Elderly - own home NESTI	%ARfD	NESTI max	%ARfD max	Elderly - residential NESTI	%ARfD
Apples	0,40		0,01032	3,3			0,00846	2,7			0,00708	2,3			0,00532	1,7			0,00529	1,7
Wine	0,54	0,1	0,00030	0,1			0,00080	0,3			0,00091	0,3			0,00056	0,2			0,00014	0,0
Wine grapes	0,54		0,00381	1,2			0,01023	3,3			0,01155	3,7			0,00707	2,3			0,00181	0,6
Table grapes	0,54		0,01956	6,3			0,00977	3,2			0,01650	5,3			0,00609	2,0			0,00435	1,4

Open point 3.17 :

Apples :
RMS agrees with that remark although considering the EU guideline, the dose in terms of kg a.s./ha is the key parameter for the acceptability of the residue trials.
Other trials (SE) with a spray concentration of 0.0045 kg a.s./hL (within 25 % of that of c GAP) should be accepted :
-parent myclobutanil : 0.043-0.07 mg/kg
-metabolite RH-9090 expressed as myclobutanil : 2 x <0.01 mg/kg

Grapes :
Other trials (SE) with a spray concentration of 0.00375 kg a.s./hL should be accepted :
-parent myclobutanil : 0.03-0.03 mg/kg
-metabolite RH-9090 expressed as myclobutanil : 2 x <0.01 mg/kg

These residue data do not change the MRL proposals on grapes and apples.

The summary sheets are given here after.

Active substance (common name):	**Myclobutanil**	Commercial Product (name):	**Systhane 20EW**
Crop/crop group:	**Apple / Pome Fruit**		
Responsible body for reporting (name & address):	Dow AgroSciences European Development Centre 2nd Floor, 3 Milton Park Abingdon, Oxon. OX14 4RN, UK	Producer of commercial product	**Dow AgroSciences**
Country:	France	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/l):	200 g/L	Other active substance in the formulation (common name and content):	None
Formulation (e.g. WP):	EW	Residues calculated as:	Myclobutanil, RH-9090 (mg/kg)
	IIA 6.3.1/04	Masterfile Reference:	ER R97.1

1 Report No. Location (region)	2 Commodity /Variety	3 Date of 1) Sowing or Planting 2) Flowering 3) Harvest	4 Method of Treatment	5 Application rate per treatment	6 Dates of Treatment(s) or No. of treatment(s) and last date	7 Growth stage at last treatment or date	8 Portion analysed (a)	9 Residues (mg/kg)	10 PHI (days)	11 Remarks:

	(a)	(b)	(c)	kg a.s./hL	Water (L/ha)	kg a.s./ha	(d)	(e)		Parent : Myclobutanil	Metabolite: RH-9090	(f)	(g)
ER R97.1 Le Terrier, 17250 St. Porchaire, France (SZ)	Apples - Golden Delicious	1) 1982 3) 12-09-96	High volume spray – almost to run-off; Knapsack sprayer	**0.0045** **0.0045** **0.0045** **0.0045** **0.0045**	917 906 848 858 794 833	**0.0413** 0.0408 0.0382 0.0386 0.0357 0.0375	6 treatments: 09-07-96 19-07-96 29-07-96 08-08-96 19-08-96 29-08-96	BBCH 85 Advanced Ripening	Whole fruit	0.04 0.04	ND <0.01	0 14	Trial No. AP/3201/HL/1F Analytical method: 310-84-13; LOQ (both analytes) = 0.01 mg/kg; Sample to analysis interval ≤ 262 days

1 Report No. Location (region)	2 Commodity /Variety (a)	3 Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	4 Method of Treatment (c)	5 Application rate per treatment kg a.s./hL	Water (L/ha)	kg a.s./ha	6 Dates of Treatment(s) or No. of treatment(s) and last date (d)	7 Growth stage at last treatment or date (e)	8 Portion analysed (a)	9 Residues (mg/kg) Parent : Myclobutanil	Metabolite: RH-9090	10 PHI (days) (f)	11 Remarks: (g)
ER R96.8 Le Terrier, 17250 St. Porchaire, France (SZ)	Apples - Golden Delicious	1) 1982 3) 12-09-96	High volume spray – almost to run-off; Knapsack sprayer	**0.0045** **0.0045** **0.0045** **0.0045** **0.0045** **0.0045**	896 906 833 875 883 833	**0.0403** **0.0408** **0.0375** **0.0394** **0.0397** 0.0375	6 treatments: 09-07-96 19-07-96 29-07-96 08-08-96 19-08-96 29-08-96	BBCH 85 Advanced Ripening	Whole fruit	0.08 0.05	<0.01 <0.01	0 14	Trial No. AP/3200/HL/1F Analytical method: 310-84-13; LOQ (both analytes) = 0.01 mg/kg; Sample to analysis interval ≤ 257 days

1 Report No. Location (region)	2 Commodity /Variety (a)	3 Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	4 Method of Treatment (c)	5 Application rate per treatment			6 Dates of Treatment(s) or No. of treatment(s) and last date (d)	7 Growth stage at last treatment or date (e)	8 Portion analysed (a)	9 Residues (mg/kg)		10 PHI (days) (f)	11 Remarks: (g)
				kg a.s./hL	Water (L/ha)	kg a.s./ha				Parent : Myclobutanil	Metabolite: RH-9090		
ER R100.4 Collebeato (BS), Lombardia, Italy (SZ)	Apples - Golden Delicious	1) 1989 3) 15-09-97	High volume spray – almost to run-off; Knapsack sprayer	**0.0045** **0.0045** **0.0045** **0.0045** **0.0045** **0.0045**	1165 1200 1200 1200 1458 1479	**0.0522** **0.0540** **0.0540** **0.0540** **0.0675** **0.0675**	6 treatments: 30-06-97 11-07-97 23-07-97 04-08-97 18-08-97 01-09-97	BBCH 85 Advanced Ripening	Whole fruit	0.08 0.07	<0.01 <0.01	0 <u>14</u>	Trial No. RAS/21/1/I Analytical method: TR 310-84-13; LOQ (both analytes) = 0.01 mg/kg; Sample to analysis interval ≤ 178 days

1 Report No. Location (region)	2 Commodity /Variety (a)	3 Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	4 Method of Treatment (c)	5 Application rate per treatment			6 Dates of Treatment(s) or No. of treatment(s) and last date (d)	7 Growth stage at last treatment or date (e)	8 Portion analysed (a)	9 Residues (mg/kg)		10 PHI (days) (f)	11 Remarks: (g)
				kg a.s./hL	Water (L/ha)	kg a.s./ha				Parent : Myclobutanil	Metabolite: RH-9090		

ER R 96.7 St. Porchaire, France (SZ)	Apples - Golden Delicious	1) 1982 3) 12-09-96	High volume spray – almost to runoff- Knapsack Sprayer	**0.0045** **0.0045** **0.0045** **0.0045** **0.0045** **0.0045**	781 867 813 860 821 813	**0.035** **0.039** **0.037** **0.039** **0.037** **0.037**	6 treatments: 09-07-96 19-07-96 29-07-96 08-08-96 19-08-96 29-08-96	BBCH 85 Fruit Ripening	Whole fruit	0.05 0.04	<0.01 <0.01	0 14	Trial No. R&H 214/1/F Analytical method: 310-84-13 LOQ (both analytes) = 0.01 mg/kg Sample to analysis interval ≤ 232 days

1 Report No. Location (region)	2 Commodity /Variety (a)	3 Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	4 Method of Treatment (c)	5 Application rate per treatment kg a.s./hL	Water (L/ha)	kg a.s./ha	6 Dates of Treatment(s) or No. of treatment(s) and last date (d)	7 Growth stage at last treatment or date (e)	8 Portion analysed (a)	9 Residues (mg/kg) Parent : Myclobutanil	Metabolite: RH-9090	10 PHI (days) (f)	11 Remarks: (g)
ER R 97.2 St. Porchaire, France (SZ)	Apples - Golden Delicious	1) 1982 3) 12-09-96	High volume spray – almost to runoff- Knapsack Sprayer	**0.0045** **0.0045** **0.0045** **0.0045** **0.0045** **0.0045**	885 881 833 869 792 833	**0.040** **0.040** **0.038** **0.039** **0.036** **0.038**	6 treatments: 09-07-96 19-07-96 29-07-96 08-08-96 19-08-96 29-08-96	BBCH 85 Fruit Ripening	Whole fruit	0.07 0.04	<0.01 nd	0 14	Trial No. R&H 217/1/F Analytical method: 310-84-13 LOQ (both analytes) = 0.01 mg/kg Sample to analysis interval ≤ 232 days

Active substance (common name): **Myclobutanil**
Crop/crop group: **Grapes**

Commercial Product (name): **Systhane 20EW**

Responsible body for reporting (name & address):	Dow AgroSciences European Development Centre 2nd Floor, 3 Milton Park Abingdon, Oxon. OX14 4RN, UK	Producer of commercial product	**Dow AgroSciences**
Country:	France	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/l):	200 g/L	Other active substance in the formulation (common name and content):	None
Formulation (e.g. WP):	EW	Residues calculated as:	Myclobutanil, RH-9090 (mg/kg)
	IIA 6.3.2/04	Masterfile Reference:	ER R95.6

1 Report No. Location (region)	2 Commodity/ Variety (a)	3 Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	4 Method of Treatment (c)	5 Application rate per treatment			6 Dates of Treatment(s) or No. of treatment(s) and last date (d)	7 Growth stage at last treatment or date (e)	8 Portion analysed (a)	9 Residues (mg/kg)		10 PHI (days) (f)	11 Remarks: (g)
				kg a.s./hL	Water (L/ha)	kg a.s./ha				Parent : Myclobutanil	Metabolite: RH-9090		
ER R95.6 17520, St. Pierre Archiac, France (SZ)	Grapes - Ungi-Blanc	1) 1981 3) 26-09-96	High volume spray – sprayed almost to runoff; Knapsack sprayer	**0.00375** **0.00375** **0.00375** **0.00375** **0.00375** **0.00375**	930 827 913 867 927 880	0.0349 0.0310 0.0342 0.0325 0.0348 0.0330	6 treatments: 22-07-96 01-08-96 12-08-96 22-08-96 02-09-96 12-09-96	BBCH 85 Fruit ripening	Whole Fruit	0.04 0.03	<0.01 <0.01	0 <u>14</u>	Trial No. AP/3198/HL/1F Analytical method: TR 310-84-13; LOQ (both analytes) = 0.01 mg/kg, Sample to analysis interval ≤ 177 days

1 Report No. Location (region)	2 Commodity/ Variety	3 Date of 1) Sowing or Planting 2) Flowering 3) Harvest	4 Method of Treatment	5 Application rate per treatment	6 Dates of Treatment(s) or No. of treatment(s) and last date	7 Growth stage at last treatment or date	8 Portion analysed (a)	9 Residues (mg/kg)	10 PHI (days)	11 Remarks:

	(a)	(b)	(c)	kg a.s./hL	Water (L/ha)	kg a.s./ha	(d)	(e)		Parent : Myclobutanil	Metabolite: RH-9090	(f)	(g)
ER R95.6 82290, La Ville Dieu du Temple, France (SZ)	Grapes - Syrah	1) 1982 3) 17-09-96	High volume spray – sprayed almost to runoff; Knapsack sprayer	**0.00375** **0.00375** **0.00375** **0.00375** **0.00375** **0.00375**	647 733 737 753 753 787	0.0243 0.0275 0.0276 0.0282 0.0282 0.0295	6 treatments: 08-07-96 18-07-96 29-07-96 09-08-96 22-08-96 03-09-96	BBCH 85 Fruit ripening	Whole Fruit	0.06 0.03	<0.01 <0.01	0 14	Trial No. AP/3198/HL/2F Analytical method: TR 310-84-13; LOQ (both analytes) = 0.01 mg/kg, Sample to analysis interval ≤ 177 days

1 Report No. Location (region)	2 Commodity/ Variety (a)	3 Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	4 Method of Treatment (c)	5 Application rate per treatment kg a.s./hL	Water (L/ha)	kg a.s./ha	6 Dates of Treatment(s) or No. of treatment(s) and last date (d)	7 Growth stage at last treatment or date (e)	8 Portion analysed (a)	9 Residues (mg/kg) Parent : Myclobutanil	Metabolite: RH-9090	10 PHI (days) (f)	11 Remarks: (g)
ER R95.5 17520, St. Pierre Archiac, France (SZ)	Grapes - Ungi-Blanc	1) 1981 3) 26-09-96	High volume spray – sprayed almost to runoff; Knapsack sprayer	**0.00375**	883 853 773 1000 1000 967	0.0331 0.0320 0.0290 0.0375 0.0375 0.0363	6 treatments: 22-07-96 01-08-96 12-08-96 22-08-96 02-09-96 12-09-96	BBCH 85 Fruit ripening	Whole Fruit	0.06 0.04	nd <0.01	0 14	Trial No. AP/3197/HL/1F Analytical method: TR 310-84-13; LOQ (both analytes) = 0.01 mg/kg, Sample to analysis interval ≤ 177 days

1	2	3	4	5	6	7	8	9	10	11

Report No. Location (region)	Commodity/ Variety (a)	Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	Method of Treatment (c)	Application rate per treatment			Dates of Treatment(s) or No. of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed (a)	Residues (mg/kg)		PHI (days) (f)	Remarks: (g)
				kg a.s./hL	Water (L/ha)	kg a.s./ha				Parent : Myclobutanil	Metabolite: RH-9090		
ER R95.5 82290, La Ville Dieu du Temple, France (SZ)	Grapes - Syrah	1) 1982 3) 17-09-96	High volume spray – sprayed almost to runoff; Knapsack sprayer	**0.00375**	680 740 770 767 810 783	0.0255 0.0278 0.0289 0.0288 0.0304 0.0294	6 treatments: 08-07-96 18-07-96 29-07-96 09-08-96 22-08-96 03-09-96	BBCH 85 Fruit ripening	Whole Fruit	0.05 0.03	nd <0.01	0 <u>14</u>	Trial No. AP/3197/HL/2F Analytical method: TR 310-84-13; LOQ (both analytes) = 0.01 mg/kg, Sample to analysis interval ≤ 177 days

1 Report No. Location (region)	2 Commodity/ Variety (a)	3 Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	4 Method of Treatment (c)	5 Application rate per treatment			6 Dates of Treatment(s) or No. of treatment(s) and last date (d)	7 Growth stage at last treatment or date (e)	8 Portion analysed (a)	9 Residues (mg/kg)		10 PHI (days) (f)	11 Remarks: (g)
				kg a.s./hL	Water (L/ha)	kg a.s./ha				Parent : Myclobutanil	Metabolite: RH-9090		
ER R96.1 17520, St. Pierre Archiac, France (SZ)	Grapes - Ungi-Blanc	1) 1981 3) 26-09-96	High volume spray – sprayed almost to runoff; Knapsack sprayer	**0.00375**	793 927 833 947 907 910	0.0297 0.0348 0.0312 0.0355 0.0340 0.0341	6 treatments: 22-07-96 01-08-96 12-08-96 22-08-96 02-09-96 12-09-96	BBCH 85 Fruit ripening	Whole Fruit	0.06 0.02	nd nd	0 <u>14</u>	Trial No. AP/3196/HL/1F Analytical method: TR 310-84-13; LOQ (both analytes) = 0.01 mg/kg, Sample to analysis interval ≤ 177 days

1 Report No. Location (region)	2 Commodity/ Variety (a)	3 Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	4 Method of Treatment (c)	5 Application rate per treatment			6 Dates of Treatment(s) or No. of treatment(s) and last date (d)	7 Growth stage at last treatment or date (e)	8 Portion analysed (a)	9 Residues (mg/kg)		10 PHI (days) (f)	11 Remarks: (g)
				kg a.s./hL	Water (L/ha)	kg a.s./ha				Parent : Myclobutanil	Metabolite: RH-9090		
ER R96.1 82290, La Ville Dieu du Temple, France (SZ)	Grapes - Syrah	1) 1982 3) 17-09-96	High volume spray – sprayed almost to runoff; Knapsack sprayer	**0.00375**	733 747 743 733 800 750	0.0275 0.0280 0.0279 0.0275 0.0300 0.0281	6 treatments: 08-07-96 18-07-96 29-07-96 09-08-96 22-08-96 03-09-96	BBCH 85 Fruit ripening	Whole Fruit	0.04 0.03	<0.01 <0.01	0 14	Trial No. AP/3196/HL/2F Analytical method: TR 310-84-13; LOQ (both analytes) = 0.01 mg/kg, Sample to analysis interval ≤ 177 days

Open point 3.23 :

1) No soil metabolites including 1,2,4-triazole were detected.

2) **B.7.9 Residues in succeeding or rotational crops in the DAR-Conclusion :**
The planting of succeeding crops is not relevant in this case since both apples and grapes are long-lived crops that are not grown in rotation with other succeeding crops.
~~Moreover, studies in rotational crops are not required since the following DT_{90} values of Myclobutanil are :~~
~~- > 1 year in field degradation studies,~~
~~- 637 to 1906 days in laboratory.~~

Open point 3.25 :

- Storage Stability Study : RH-3866 (myclobutanil fungicide) and RH-9090 in Almond Meat and Hulls (Batra R., 1997a)

Table B.7.14-4 (DAR): Percent recovery for method recovery test (%).

Plant commodity	Storage duration interval (months)	Fortification level (mg/kg)		Average percent recovery	
		RH-3866 parent	RH-9090	RH-3866 parent	RH-9090
Almond meat	**0**	1	1	95.9	83.7
	3			84.4	71.6
	6			99.2	83.4
	12			89.5	79.2
	18			**127**	87.1
	24			71.7	**59.9**
Average				95.3	76.9
Standard deviation				20.3	11.1
Almond hulls	**0**	1	2	98.2	133
	3			91.0	91.9
	6			88.4	85.7
	12			113	66.3
	18			108	71.3
	24			**67.1**	**66.5**
Average				94.2	83.4
Standard deviation				17.3	24.7

Table B.7.14-5 (DAR) : Percent recovery in samples of almond meat and hulls found in the course of the frozen storage stability study – corrected for the mean of concurrent recoveries.

Plant commodity	Test substances	Storage period (months)					
		0	**3**	**6**	**12**	**18**	**24**
Almond meat	**RH-3866 parent**	109	96.3	97.6	92.3	102	92.2
	RH-9090	107	95.3	95.6	90.6	86.5	85.3
Almond hulls	**RH-3866 parent**	92.4	104	105	96.4	83.5	103
	RH-9090	86.7	83.9	88.1	105	80.9	91.3

Conclusion :
~~Recovery values (presented in the table here above) were generally acceptable both for the RH-3866 parent and its metabolite RH-9090.~~

The results for 24 months for almond hulls and meat are not acceptable for both the parent and the metabolite RH-9090 since the residues levels corrected for procedural recoveries were < 70%.
It is more appropriate to assign a storage stability of 18 months in both cases although the samples that are corrected for the concurrent recoveries do not show any significant decline for the 24 month time point.
The residues of myclobutanil and its metabolite RH-9090 are considered as stable in almond meat and hulls for up to 24 months (after storage at –10°C).

ANNEX B
(Adeendum March 2007)

Myclobutanil

B.8 Environmental fate and behaviour

B.8.6.1 Predicted environmental concentrations in ground water (PECgw) (Annex IIIA 9.21)

Modelling the leaching of myclobutanil and a potentially relevant metabolite (β-4-chlorophenyl-β-cyano-γ-(1H 1,2,4-triazole)butyric acid) to groundwater in the EU using PEARL and the FOCUS scenarios (Reeves, G., 2006)

Guideline :
According to guidance given in "FOCUS groundwater scenarios in the EU pesticide registration process". Report of the FOCUS Groundwater Scenarios Workgroup,
EC Document Reference Sanco/321/2000 rev.2, 202pp.
Method of calculation:
The PECgw have been recalculated according to FOCUS PEARL (ver. 3.3.3.) as a second modelling software.
The "tier 1"PEARL calculations have been performed according the assumptions that were considered for the previous PELMO PEC calculations: GAPs, choice of endpoints.
The "higher tier" PEARL calculations have been performed according to the same assumptions that were considered for the previous PELMO PEC. However, worst case field DT50 standardised at soil temperature at 20°C has been considered.

"The use of a field DT50 for higher tier modelling is a recognised approach where it can be justified, as referenced by the FOCUS kinetics work group (2006). It is considered valid in this case because the DT50 is determined under conditions more specific to the intended use of myclobutanil in an agricultural field, i.e. unsieved soil, fluctuating soil and moisture conditions and importantly, the availability of a sustainable biomass to facilitate microbial degradation, which is often lacking when a soil is maintained under laboratory conditions. Also, since myclobutanil is not considered volatile or that it has a low Koc, then the decline under field conditions is considered due to degradation and not dissipation."

The DT50 field was derived by normalising individual values from 4 North European dissipation trials for temperature (20°C) using a day-length normalisation approach (correction of the day length in function of difference between the observed daily temperature and the reference temperature of 20°C). No correction was feasible for soil moisture. The soil moisture standardisation would only have the effect of reducing the day-length and so this represents a worst case for the resultant DT50 field. Kinetic analysis (first order kinetics, non-log transformed data) was then carried out using the data (cumulative Day After Treatment versus myclobutanil soil concentration). The worst case field DT50 was considered for the PECgw calculation.

	DT_{50}	DT_{90}	R^2
Schwanheim	7	24	0.736
Stelle	60	199	0.696
Gersthofen	54	179	0.322
Bornheim	10	33	0.776

Worst case and realistic case scenarios were applied (minor differences in terms of application timing and crop interception – both types of scenarios give similar PEC results).

Application of Systhane 20EW to apples (4 x 90 g as/ha)

Worst case

Appn. 1 15 Apr, 65% crop intercept (flowering), effective rate 31.5 g as/ha
Appn. 2 25 Apr, 65% crop intercept (flowering), effective rate 31.5 g as/ha
Appn. 3 5 May, 70% crop intercept (foliage development), effective rate 27 g as/ha
Appn. 4 15 May, 70% crop intercept (foliage development), effective rate 27 g as/ha

Realistic case

Appn. 1 15 Apr, 65% crop intercept (flowering), effective rate 31.5 g as/ha
Appn. 2 31 May, 70% crop intercept (foliage development), effective rate 27 g as/ha

Appn. 3 20 Jun, 70% crop intercept (foliage development), effective rate 27 g as/ha
Appn. 4 1 Jul, 80% crop intercept (full foliage), effective rate 18 g as/ha

Application of Systhane 20EW to vines (4 x 48 g as/ha)

Worst case

Appn. 1 15 May, 60% crop intercept (leaf development), effective rate 19.2 g as/ha
Appn. 2 25 May, 70% crop intercept (flowering), effective rate 14.4 g as/ha
Appn. 3 4 Jun, 70% crop intercept (flowering), effective rate 14.4 g as/ha
Appn. 4 14 Jun, 70% crop intercept (flowering), effective rate 14.4 g as/ha

Realistic case

Appn. 1 20 Jun, 70% crop intercept (flowering), effective rate 14.4 g as/ha
Appn. 2 30 Jun, 70% crop intercept (flowering), effective rate 14.4 g as/ha
Appn. 3 10 Jul, 70% crop intercept (flowering), effective rate 14.4 g as/ha
Appn. 4 20 Jul, 85% crop intercept (ripening), effective rate 7.2 g as/ha

The other input parameters that are not mentioned in the table here below were chosen as default.

Method of calculation and type of study (*e.g.* modeling, monitoring, lysimeter)	<u>For FOCUS gw modelling, values used –</u> Modelling using FOCUS model(s), with appropriate FOCUS gw scenarios, according to FOCUS guidance. Model(s) used: FOCUSPEARL 3.3.3 Scenarios: Chateaudun, Hamburg, Jokioinen, Kremsmünster, Okehampton, Piacenza, Porto, Sevilla, Thiva Crop: apples and vines <u>a.s</u> Geometric mean parent DT_{50lab} 250 d (normalisation to 10kPa or pF2, 20°C with Q10 of 2.2) used for the "Tier 1" calculation Worst case field DT_{50} 60 d (normalisation to 20°C with Q10 of 2.2) used for the "Higher tier" calculation K_{foc}: parent, mean or median 517 mL/g (or Kom = 301 mL/g, $^1/_n$= 0.88. Molecular weight : 288.8 Vapour pressure: 1.98 10^{-4} Pa Water solubility 132 mg/L <u>'butyric acid' metabolite'</u> Geometric mean DT_{50lab} 10 d (normalisation to 10kPa or pF2, 25°C). K_{foc}: mean 36 mL/g (or Kom = 21 mL/g), $^1/_n$= 09. Molecular weight : 288.8 Vapour pressure: not applicable Water solubility not applicable Fraction formed: 6%
Application rate	Apples Application rate: 90 g/ha. No. of applications: 4 Time of application): 15 April to 1 July Vines Application rate: 48 g/ha. No. of applications: 4 Time of application): 20 June to 20 July

Findings :

PEC$_{(gw)}$

Maximum concentration

Average annual concentration

(Results quoted for modelling with FOCUS gw scenarios, according to FOCUS guidance.)

-
Annual average concentrations (80th percentile) according to FOCUS guidance: FOCUSPEARL - "Tier 1" PEC using geomean standardised (for temperature and moisture) DT_{50} lab active substance: <0.001 - 1.160 µg/L butyric acid metabolite: <0.001- 0.043 µg/L FOCUSPEARL - "Higher tier" PEC using worst case standardised (for temperature) DT_{50} field active substance: <0.001 - 0.001 µg/L butyric acid metabolite: <0.001 - 0.012 µg/L (see detailed results in table below)

FOCUSPEARL - "Tier 1" PEC using geomean standardised (for temperature and moisture) DT_{50} lab - 80th percentile annual average leachate concentration /L)

Scenarios		Chateaudun	Hamburg	Jokioinen	Kremsmünster	Okehampton	Piacenza	Porto	Sevilla	Thiva
Apples (worst case)	a.s.	0.453	0.420	0.002	0.315	0.344	1.160	<0.001	0.268	0.479
	Met.	0.020	0.027	0.005	0.014	0.020	0.043	<0.001	0.015	0.018
Apples (realistic case)	a.s.	0.378	0.355	0.001	0.263	0.288	1.004	<0.001	0.220	0.406
	Met.	0.017	0.023	0.004	0.012	0.017	0.017	<0.001	0.012	0.015
Vines (worst case)	a.s.	0.209	0.123	-	0.100	-	0.517	<0.001	0.109	0.205
	Met.	0.010	0.009	-	0.005	-	0.021	<0.001	0.007	0.008
Vines (realistic case)	a.s.	0.153	0.089	-	0.074	-	0.405	<0.001	0.077	0.152
	Met.	0.008	0.007	-	0.004	-	0.016	<0.001	0.005	0.006

FOCUSPEARL - "Higher tier" PEC using worst case standardised (for temperature) DT_{50} field- 80th percentile annual average leachate concentration /L)

Scenarios		Chateaudun	Hamburg	Jokioinen	Kremsmünster	Okehampton	Piacenza	Porto	Sevilla	Thiva
Apples (worst case)	a.s.	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001	< 0.001	< 0.001	< 0.001
	Met.	0.002	0.004	0.001	0.002	0.004	0.012	<0.001	0.001	0.002
Apples (realistic case)	a.s.	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001	< 0.001	< 0.001	< 0.001
	Met.	0.002	0.004	0.001	0.002	0.003	0.012	< 0.001	0.001	0.001
Vines (worst case)	a.s.	< 0.001	< 0.001	-	< 0.001	-	0.001	<0.001	< 0.001	< 0.001

	Met.	0.001	0.001	-	< 0.001	-	0.006	< 0.001	0.001	0.001
Vines (realistic case)	a.s.	< 0.001	< 0.001	-	< 0.001	-	< 0.001	< 0.001	< 0.001	< 0.001
	Met.	0.001	0.001	-	< 0.001	-	< 0.001	< 0.001	< 0.001	< 0.001

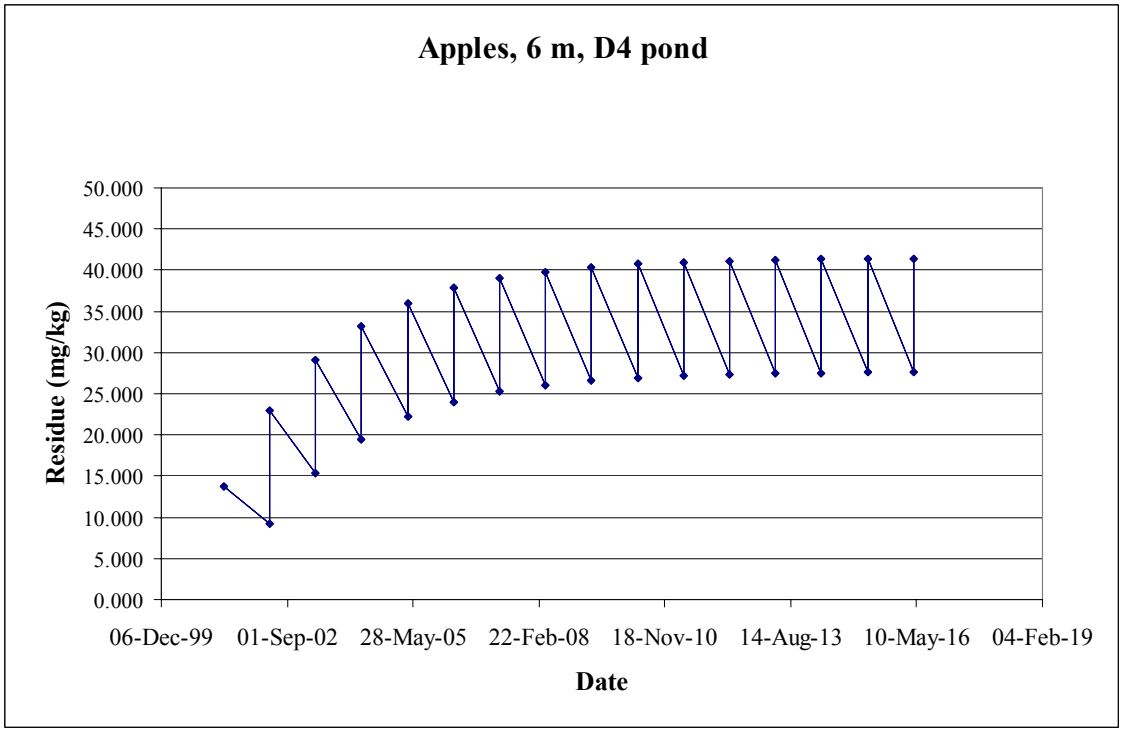
Conclusions:
The PEC gw calculations have been made by means of 2 modelling software (FOCUSPELMO considering laboratory DT50 and FOCUSPEARL considering laboratory and field DT50).
The PEC calculations indicate that the risk to groundwater (a.s. and butyric acid metabolite) is acceptable for most of the scenarios.

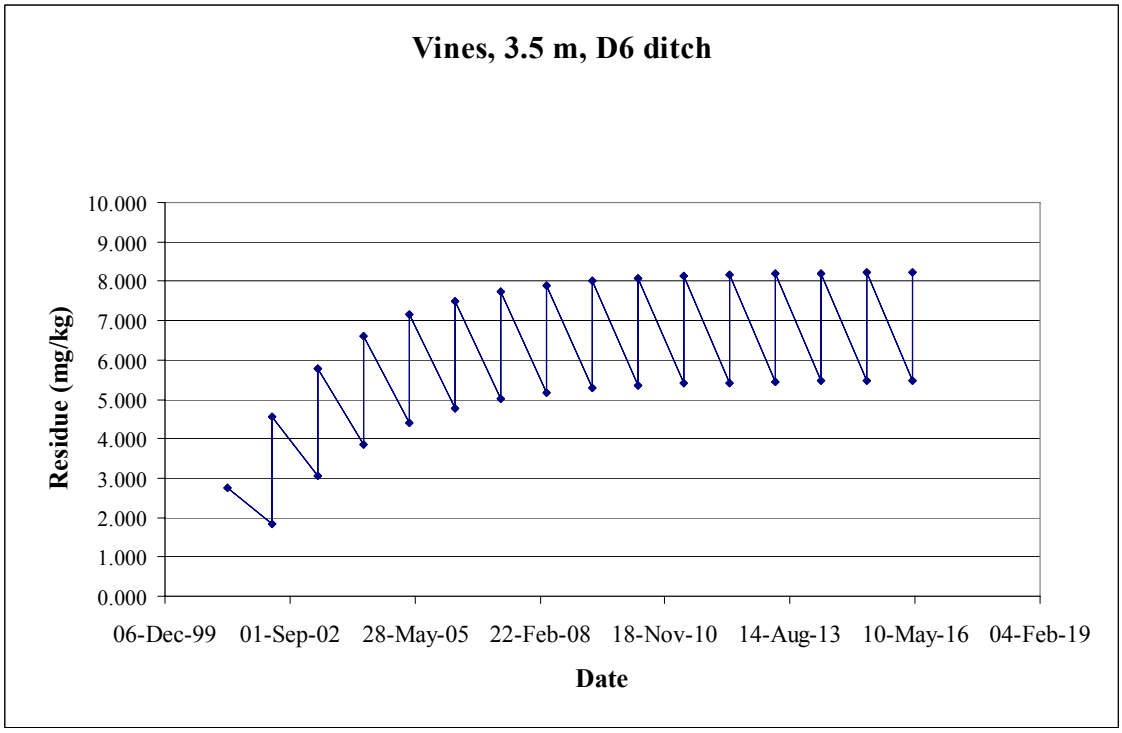
B.8.6.2 Predicted environmental concentrations in sediment (PECsed) (Annex IIIA 9.2.3)

The PEC have been recalculated for accumulation in sediment in consecutive years.

FOCUSsw is not set up to take into account pesticide applications in consecutive years, and so this tool is not currently considered appropriate to derive an "accumulation" PEC_{SED}. However, this can be estimated using a spreadsheet and the procedure is described below. It should be noted that this was the same procedure used to derive the plateau concentration for soil under Annex IIIA, Point 9.1.3.

A worst case approach was adopted whereby the global maximum PEC_{SED} derived from the multiple application FOCUSsw analysis was used as the starting concentration. For apples, this was the D4 pond at 6 m, i.e. 13.791 µg/kg, and for vines this was the D6 ditch at 3.5 m, i.e. 2.738 µg/kg. It was assumed that these were the starting concentrations after year 1, and that subsequent applications in consecutive years added the same loading to the sediment, except that some degradation would occur with a DT_{50}(sediment) of 626 days. The results are presented graphically as follows:





In both cases it is predicted that myclobutanil will not accumulate in sediment.

Conclusions:
The PEC sediment have been calculated assuming that

- the global maximum PEC_{SED} derived from the multiple application FOCUSsw analysis is the starting concentration
- A worst case DT50sediment of 626 days derived from the w/s study.

The sediment accumulation modelling showed that for apples use, the predicted maximum myclobutanil PEC_{SED} was *ca* 41 µg/kg, whilst for vines use, the predicted maximum myclobutanil PEC_{SED} was *ca* 8.2 µg/kg.

B.8.6.2 Predicted environmental concentrations in surface water (PECsw) (Annex IIIA 9.2.3)

Step 3 and 4 FOCUSsw simulations have been carried out for a single application. The simulations used the same input data as for the multiple applications reported previously but with the following exceptions. Firstly, a more correct (according to current FOCUS guidance) geomean soil DT_{50} of 250 days was used, rather than the arithmetic mean of 284 days used previously, and secondly, 1 x 48 g as/ha was selected, rather than 4 x 48 g as/ha as before. For apples at Step 4, a 14 m no-spray zone was selected. The results for the single application are shown in the following tables.

Step 3 (Default No-spray Zones), Single Application, Apples – Early

Surface Water (µg/L)

Location	Water body	Global Max	TWA 1d	TWA 2d	TWA 4d	TWA 7d	TWA 14d	TWA 21d	TWA 28d	TWA 42d	TWA 50d	TWA 100d
D3	ditch	6.980	5.400	3.497	1.820	1.049	0.528	0.353	0.265	0.177	0.149	0.0746
D4	pond	0.445	0.437	0.432	0.424	0.414	0.397	0.385	0.380	0.370	0.364	0.330
D4	stream	6.696	0.391	0.326	0.302	0.274	0.226	0.219	0.201	0.155	0.141	0.0837
D5	pond	0.499	0.492	0.487	0.478	0.469	0.452	0.439	0.427	0.407	0.397	0.351
D5	stream	7.337	0.499	0.253	0.130	0.0824	0.0658	0.0551	0.0477	0.0384	0.0352	0.0271
R1	pond	0.424	0.417	0.412	0.403	0.393	0.374	0.359	0.345	0.323	0.312	0.257
R1	stream	5.648	0.977	0.489	0.245	0.140	0.0700	0.0467	0.0387	0.0299	0.0251	0.0143
R2	stream	7.494	0.649	0.325	0.163	0.0929	0.0465	0.0310	0.0233	0.0155	0.0182	0.0091
R3	stream	7.991	2.582	1.297	0.651	0.531	0.266	0.178	0.133	0.0890	0.0748	0.0374
R4	stream	5.682	1.153	0.577	0.289	0.165	0.116	0.0777	0.0710	0.0587	0.0493	0.0254

Sediment (µg/kg dry weight)

Location	Water body	Global Max	TWA 1d	TWA 2d	TWA 4d	TWA 7d	TWA 14d	TWA 21d	TWA 28d	TWA 42d	TWA 50d	TWA 100d
D3	ditch	3.194	3.092	2.865	2.422	1.995	1.512	1.271	1.120	0.934	0.863	0.626
D4	pond	3.998	3.998	3.998	3.998	3.998	3.997	3.997	3.996	3.993	3.990	3.923
D4	stream	0.893	0.892	0.892	0.889	0.883	0.870	0.848	0.822	0.797	0.788	0.696
D5	pond	3.305	3.305	3.305	3.305	3.305	3.304	3.303	3.302	3.297	3.294	3.239
D5	stream	0.512	0.453	0.409	0.358	0.318	0.276	0.266	0.257	0.247	0.247	0.224
R1	pond	1.903	1.903	1.903	1.903	1.902	1.902	1.901	1.900	1.897	1.895	1.878
R1	stream	0.665	0.566	0.481	0.382	0.306	0.227	0.189	0.173	0.159	0.151	0.127
R2	stream	0.451	0.377	0.321	0.254	0.203	0.151	0.126	0.114	0.103	0.0981	0.0921
R3	stream	1.594	1.413	1.209	0.961	0.932	0.785	0.677	0.603	0.510	0.473	0.348
R4	stream	0.773	0.662	0.563	0.447	0.358	0.311	0.285	0.282	0.283	0.274	0.219

Step 4 (14 m No-spray Zones), Single Application, Apples – Early

Surface Water (µg/L)

Location	Water body	Global Max	TWA 1d	TWA 2d	TWA 4d	TWA 7d	TWA 14d	TWA 21d	TWA 28d	TWA 42d	TWA 50d	TWA 100d
D3	ditch	1.781	1.376	0.890	0.463	0.267	0.134	0.0899	0.0675	0.0451	0.0379	0.0190
D4	pond	0.349	0.349	0.349	0.348	0.347	0.344	0.340	0.335	0.327	0.322	0.290
D4	stream	1.874	0.354	0.326	0.302	0.274	0.226	0.219	0.201	0.155	0.141	0.0837
D5	pond	0.229	0.227	0.225	0.221	0.217	0.210	0.205	0.199	0.191	0.186	0.166
D5	stream	2.050	0.144	0.110	0.0877	0.0824	0.0658	0.0551	0.0477	0.0384	0.0352	0.0236
R1	pond	0.155	0.152	0.150	0.147	0.143	0.136	0.130	0.126	0.118	0.114	0.0946
R1	stream	1.575	0.272	0.136	0.0683	0.0390	0.0196	0.0131	0.0135	0.0131	0.0110	0.00725
R2	stream	2.089	0.238	0.129	0.0644	0.0368	0.0184	0.0123	0.00923	0.00615	0.00877	0.00440
R3	stream	2.228	1.026	0.554	0.279	0.262	0.132	0.0881	0.0661	0.0441	0.0371	0.0185
R4	stream	1.584	0.469	0.236	0.119	0.0683	0.0595	0.0397	0.0467	0.0388	0.0326	0.0170

Sediment (µg/kg dry weight)

Location	Water body	Global Max	TWA 1d	TWA 2d	TWA 4d	TWA 7d	TWA 14d	TWA 21d	TWA 28d	TWA 42d	TWA 50d	TWA 100d
D3	ditch	0.859	0.834	0.777	0.663	0.549	0.418	0.352	0.310	0.259	0.239	0.173
D4	pond	-	2.981	2.981	2.981	2.981	2.979	2.977	2.974	2.963	2.955	2.848
D4	stream	0.886	0.885	0.885	0.882	0.876	0.863	0.841	0.815	0.790	0.781	0.689
D5	pond	-	2.063	2.063	2.063	2.063	2.061	2.059	2.057	2.048	2.041	1.917
D5	stream	0.289	0.288	0.287	0.285	0.279	0.268	0.258	0.249	0.239	0.240	0.216
R1	pond	0.746	0.746	0.746	0.746	0.746	0.746	0.746	0.745	0.744	0.743	0.737
R1	stream	0.188	0.163	0.141	0.120	0.106	0.0890	0.0799	0.0791	0.0756	0.0730	0.0630
R2	stream	0.202	0.185	0.169	0.147	0.128	0.105	0.0935	0.0859	0.0761	0.0721	0.0574
R3	stream	0.807	0.754	0.684	0.583	0.494	0.391	0.348	0.316	0.272	0.254	0.190
R4	stream	0.378	0.359	0.337	0.305	0.273	0.229	0.219	0.206	0.192	0.183	0.147

Step 3 (Default No-spray Zones), Single Application, Vines – Late

Surface Water (µg/L)

Location	Water body	Global Max	TWA 1d	TWA 2d	TWA 4d	TWA 7d	TWA 14d	TWA 21d	TWA 28d	TWA 42d	TWA 50d	TWA 100d
D6	ditch	0.824	0.786	0.761	0.720	0.634	0.416	0.292	0.223	0.151	0.127	0.0647
R1	pond	0.0308	0.0305	0.0303	0.0299	0.0294	0.0282	0.0277	0.0271	0.0257	0.0249	0.0208
R1	stream	0.602	0.349	0.175	0.0879	0.0503	0.0338	0.0225	0.0169	0.0113	0.00991	0.00564
R2	stream	0.806	0.161	0.0867	0.0435	0.0249	0.0125	0.0123	0.00927	0.00619	0.00520	0.00260
R3	stream	0.842	0.219	0.110	0.0552	0.0316	0.0158	0.0105	0.00792	0.00528	0.00444	0.00222
R4	stream	0.592	0.363	0.203	0.102	0.0583	0.0292	0.0195	0.0146	0.00974	0.00818	0.00524

Sediment (µg/kg dry weight)

Location	Water body	Global Max	TWA 1d	TWA 2d	TWA 4d	TWA 7d	TWA 14d	TWA 21d	TWA 28d	TWA 42d	TWA 50d	TWA 100d
D6	ditch	1.316	1.315	1.312	1.298	1.265	1.159	1.056	0.970	0.845	0.792	0.597
R1	pond	0.183	0.183	0.183	0.183	0.183	0.183	0.183	0.183	0.183	0.182	0.181
R1	stream	0.268	0.245	0.221	0.188	0.159	0.127	0.111	0.101	0.0876	0.0832	0.0680
R2	stream	0.211	0.199	0.187	0.170	0.155	0.137	0.127	0.120	0.110	0.106	0.0883
R3	stream	0.145	0.130	0.114	0.0926	0.0749	0.0561	0.0469	0.0412	0.0343	0.0316	0.0227
R4	stream	0.260	0.241	0.217	0.183	0.154	0.121	0.104	0.0935	0.0803	0.0751	0.0630

Conclusions:
The PECsw and PEC sed for single application pattern have been calculated considering the assumptions used for the previous PEC calculations. Considering the very high uncertainty related to the FOCUS PEC surface water simulations, the results of both PEC calculations (single or multiple applications) are similar. We consider therefore that it is more appropriate to base the TER calculations on the PEC multiple applications. Moreover, the risk assessment shows that the risk for aquatic organisms is acceptable with rather easily feasible mitigations measures (short bufferzones)

Draft Assessment Report

ADDENDUM

March 2007

Myclobutanil

Volume 4

Confidential information

Rapporteur Member State: Belgium

CONFIDENTIAL BUSINESS INFORMATION:

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ANNEX B

Myclobutanil

B.9 Ecotoxicology
(Update March 2007)

B.9.1 Effects on birds (Annex IIA 8.1; Annex IIIA 10.1)

B.9.1.2 Avian dietary toxicity (5 day) (Annex IIA 8.1.2)

8-Day Dietary LC_{50} Study with RH-53,866 Technical in Bobwhite Quail. (Fletcher D.W., 1984a).

Guidelines :
FIFRA 71-1, ASTM Standard E857-81, equivalent to OECD 205, "Avian Dietary Toxicity Test"
GLP :
Yes
Material and Methods :
Test substance : myclobutanil, chemical purity: 84.5 %, batch n°: LSPL 83/0017E
Test species : bobwhite quail, *Colinus virginianus*
Sex, weight, age : 10 birds per treatment, 50 birds for the control group, not sexed, 29.8 – 30.8 g, 13 days old
Applied concentrations :
untreated control; 312, 625, 1250, 2500, 5000 mg a.s./kg feed
The test material was dissolved in acetone before incorporation into the standard diet.
Type of application : dietary application
Time of exposure : short-term feeding test (5 days exposure period + 3 days observation period)
Test conditions :
temperature : 33 - 40 °C
relative humidity : 24 – 30 %
photoperiod : 24 hours light per day
Findings :
Mortality : No mortalities occurred in the control group and in the test groups up to 1250 mg a.s./kg feed. Mortality in the treatment groups of 2500 and 5000 mg a.s./kg feed was 20 % and 10 % respectively.
Clinical signs : No abnormal reactions or systemic signs of toxicity were noted in birds fed myclobutanil at dietary levels of 312, 625 and 1250 mg a.s./kg feed, other than slight food avoidance on test day 2. Anorexia and lethargy were noted in birds receiving myclobutanil at dietary levels of 2500 and 5000 mg a.s./kg feed.
Macroscopic post mortem examination : No abnormal tissue alterations were observed.
Body weight : Mean body weight gains on test day 5 were smaller in all treatment groups, except for the 625 mg a.s./kg feed group, compared to the control. Mean body weight gains on test day 8 were smaller in the treatment groups of 2500 and 5000 mg a.s./kg feed, compared to the control.
Feed consumption : Food consumption depressions were noted in the 2500 and 5000 mg a.s./kg feed groups during the test period compared to the control group. The food consumption in all other test groups was comparable to the control group during the test period and the observation period.
Conclusions :
The study is acceptable.
Endpoints :
LC_{50} (*Colinus virginianus*, 5 d) > 5000 mg a.s./kg feed or 567 mg a.s./kg b.w./day based on a mean food consumption of 3.7 g/bird/day and a mean body weight of 32.6 g/bird (day 0-5 at 5000 mg a.s./kg feed)

8-Day Dietary LC_{50} Study with RH-53,866 Technical in Mallard Ducklings. (Fletcher D.W., 1984b).

Guidelines :
FIFRA 71-1, ASTM Standard E857-81, equivalent to OECD 205, "Avian Dietary Toxicity Test"
GLP :
Yes
Material and Methods :
Test substance : myclobutanil, chemical purity: 84.5 %, batch n°: LSPL 83/0017E
Test species : mallard ducklings, *Anas platyrhynchos*
Sex, weight, age : 10 birds per treatment, 50 birds for the control group, not sexed, 90.8 – 99.0 g, 7 days old
Applied concentrations :
untreated control; 312, 625, 1250, 2500, 5000 mg a.s./kg feed

The test material was dissolved in acetone before incorporation into the standard diet.
Type of application : dietary application
Time of exposure : short-term feeding test (5 days exposure period + 3 days observation period)
Test conditions :
temperature : 21 - 23 °C
relative humidity : 35 – 59 %
photoperiod : 12 hours light per day
Findings :
Mortality : No mortalities occurred in the control group and in the test groups up to 2500 mg a.s./kg feed. Mortality in the treatment group of 5000 mg a.s./kg feed was 10 %.
Clinical signs : No abnormal alterations or systemic signs of toxicity were noted in birds fed myclobutanil at dietary levels of 312, 625 and 1250 mg a.s./kg feed. Anorexia and lethargy were noted in birds receiving myclobutanil at dietary levels of 2500 and 5000 mg a.s./kg feed.
Macroscopic post mortem examination : No abnormal tissue alterations were observed.
Body weight : Mean body weight gains during the test period were smaller in the 5000 mg a.s./kg feed group, compared to the control. Overall mean body weight gains during the investigation in the test and control groups were comparable.
Feed consumption : Food consumption depressions were noted in one of the control groups and in the 5000 mg a.s./kg feed group. The food consumption in all other test groups was comparable to the control group during the test period and the observation period.
Conclusions :
The study is acceptable.
Endpoints :
LC_{50} (*Anas platyrhynchos*, 5 d) > 5000 mg a.s./kg feed or 1544 mg a.s./kg b.w./day based on a mean food consumption of 31.5 g/bird/day and a mean body weight of 102 g/bird (day 0-5 at 5000 mg a.s./kg feed)

B.9.2 Effects on aquatic organisms (fish, aquatic invertebrates, algae) (Annex IIA 8.2; Annex IIIA 10.2)

B.9.2.8 Effects on algal growth (Annex IIA 8.2.6)

Acute Toxicity of Myclobutanil Technical (RH-3866) to *Scenedesmus subspicatus*. (Ellgehausen H., 1987).

Guidelines :
OECD Guideline 201 : Alga, growth inhibition test
GLP :
Yes
Material and Methods :
Test substance : myclobutanil, chemical purity: 93.0 %, batch n°: 565803
Test species : green alga, *Desmodesmus subspicatus*
Number of replicates, initial cell density : 3 replicates/treatment, 10^4 cells/mL
Type of test : 96 hours static toxicity test
Applied and measured concentrations :
nominal : control; solvent control (0.01 % acetone); 0.625, 1.25, 2.5, 5.0, 10.0 mg a.s./L
Test conditions :
temperature : 20 ± 2 °C
pH : 7.6 – 8.2
light : continuous, 8000 lux
Findings and Conclusions :
The study is acceptable.
Endpoints :
E_bC_{50} (*Desmodesmus subspicatus*, 96 h) = 2.655 mg a.s./L (based on nominal concentrations)
E_rC_{50} (*Desmodesmus subspicatus*, 72 h) = 7.5 mg a.s./L (based on nominal concentrations)
E_rC_{50} (*Desmodesmus subspicatus*, 96 h) = 6.7 mg a.s./L (based on nominal concentrations)

RH-3866 Technical – Toxicity to the Freshwater Green Alga, *Selenastrum capricornutum.* (Hoberg J.R., 1991).

Guidelines :
U.S. EPA FIFRA, 40 CFR, Part 158.150 Guidelines 122-2 and 123-2
GLP :
Yes
Material and Methods :
Test substance : myclobutanil, chemical purity: 93 %, batch n°: 2-2131
Test species : green alga, *Pseudokirchneriella subcapitata*
Number of replicates, initial cell density : 3 replicates/treatment, 0.3 x 10^4 cells/mL
Type of test : 120 hours static toxicity test
Applied and measured concentrations :
nominal : control; solvent control (acetone); 0.65, 1.3, 2.5, 5.0, 10 mg a.s./L
mean measured : control; solvent control (acetone); 0.56, 1.1, 2.2, 5.1, 6.6 mg a.s./L
measured concentrations ranging from 66 –102 % of the nominal concentrations
Test conditions :
temperature : 24 – 26 °C
pH : 7.4 – 7.5 (at start); 8.5 – 10.6 (at end)
The pH change during the test is due to photosynthesis and respiration by the algae.
light : continuous, 4304 - 5380 lux
Analytical methods : gas chromatography with electron capture detection (GC-ECD)
Findings :
Cell growth was completely inhibited at 120 hours of exposure in the three highest concentrations of myclobutanil tested (2.2, 5.1 and 6.6 mg a.s./L). These treatment levels were excluded from statistical analysis due to the obvious concentration-effect (no growth). Statistical analysis of the remaining concentrations tested

(1.1 and 0.56 mg a.s./L) demonstrated a significant reduction in cell density in the 1.1 mg a.s./L treatment level when compared to cell density of the pooled controls.
After 120 hours of exposure, there were no intact cells present in the 2.2, 5.1 and 6.6 mg a.s./L treatment levels. Few intact cells were observed in the 1.1 mg a.s./L treatment level. Cells in this treatment level were also observed to be fragmented and mishappen, with thin walls. Cells in the 0.56 mg a.s./L treatment level appeared normal in comparison with the control cultures.
Conclusions :
The study is acceptable.
Endpoints :
E_bC_{50} (*Pseudokirchneriella subcapitata*, 120 h) = 1.1 mg a.s./L
E_rC_{50} (*Pseudokirchneriella subcapitata*, 120 h) = 1.2 mg a.s./L
NOEC (*Pseudokirchneriella subcapitata*, 120 h) = 0.56 mg a.s./L

All results were based on mean measured concentrations.

Myclobutanil butyric acid soil metabolite: Growth inhibition test with the freshwater green alga, *Pseudokirchneriella subcapitata*. (Hancock G.A. *et al*., 2004).

Guidelines :
OECD Guideline 201 : Alga, growth inhibition test,
U.S. EPA FIFRA Guidelines 123-2
GLP :
Yes
Material and Methods :
Test substance : myclobutanil butyric acid, chemical purity: 98 %, batch n°: F1132-034
Test species : green alga, *Pseudokirchneriella subcapitata*
Number of replicates, initial cell density : 6 replicates for the control; 3 replicates/treatment, 10^4 cells/mL
Type of test : 96 hours static toxicity test
Applied and measured concentrations :
nominal : control; 3.13, 6.25, 12.5, 25.0, 50.0, 100 mg myclobutanil butyric acid/L
measured concentrations ranging from 95.5 –106 % of the nominal concentrations
Test conditions :
temperature : 24.0 – 24.1 °C
pH : 6.1 – 7.4 (at start); 8.4 – 10.1 (at end)
light : continuous, 7100 - 8550 lux
Analytical methods : HPLC with UV detection
Findings :
Microscopic evaluation of the cells at each test concentration and the control revealed no abnormal observations at any test level.
Conclusions :
The study is acceptable.
Endpoints :
E_bC_{50} (*Pseudokirchneriella subcapitata*, 72 h) = 68.3 mg myclobutanil butyric acid/L
NOEC (*Pseudokirchneriella subcapitata*, 72 h) = 51.5 mg myclobutanil butyric acid/L (biomass)
E_bC_{50} (*Pseudokirchneriella subcapitata*, 96 h) = 56.2 mg myclobutanil butyric acid/L
NOEC (*Pseudokirchneriella subcapitata*, 96 h) = 12.4 mg myclobutanil butyric acid/L (biomass)
E_rC_{50} (*Pseudokirchneriella subcapitata*, 72 h) = 73.6 mg myclobutanil butyric acid/L
NOEC (*Pseudokirchneriella subcapitata*, 72 h) = 51.5 mg myclobutanil butyric acid/L (growth rate)
E_rC_{50} (*Pseudokirchneriella subcapitata*, 96 h) = 69.2 mg myclobutanil butyric acid/L
NOEC (*Pseudokirchneriella subcapitata*, 96 h) = 51.5 mg myclobutanil butyric acid/L (growth rate)

All results were expressed as mean measured myclobutanil butyric acid concentrations.

B.9.2.15 Summary of effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2)

Table B.9.2.15-1 : Summary of effects of myclobutanil on aquatic organisms

Test species	Test substance	Time-scale	Endpoints	References
Oncorhynchus mykiss	myclobutanil	96 h static	**LC_{50} = 2.0 mg a.s./L** (initial)	Putt A., 2003
Lepomis macrochirus	myclobutanil	96 h static	LC_{50} = 4.1 mg a.s./L (mm)	Putt A., 2003b
Cyprinodon variegatus	myclobutanil	96 h flow-through	LC_{50} = 4.7 mg a.s./L (mm)	Sousa J.V., 1991
Oncorhynchus mykiss	myclobutanil	21 d flow-through	**NOEC = 0.2 mg a.s./L** (nom)	Ritter A., 1990
Pimephales promelas	myclobutanil	35 d flow-through	NOEC = 0.98 mg a.s./L (mm)	McAllister W. A. *et al.*, 1986
Daphnia magna	myclobutanil	48 h static	EC_{50} = 17 mg a.s./L (mm)	Putt A., 2003
Mysidopsis bahia	myclobutanil	96 h flow-through	**EC_{50} = 0.24 mg a.s./L** (mm)	Sousa J. V., 1991
Crassostrea virginica	myclobutanil	96 h flow-through	EC_{50} = 0.72 mg a.s./L (mm)	Dionne E., 1991
Daphnia magna	myclobutanil	21 d semi-static	**NOEC = 1.0 mg a.s./L** (nom)	Ritter A., 1990
Desmodesmus subspicatus	myclobutanil	96 h static	E_bC_{50} = 2.655 mg a.s./L E_rC_{50} = 6.7 mg a.s./L (nom)	Ellgehausen H., 1987
Pseudokirchneriella subcapitata	myclobutanil	120 h static	**E_bC_{50} = 1.1 mg a.s./L** E_rC_{50} = 1.2 mg a.s./L (mm)	Hoberg J. R., 1991
Chironomus riparius	myclobutanil	30 d static s/w system	**NOEC = 4.98 mg a.s./L** (mm)	van der Kolk J., 1995

Table B.9.2.15-2 : Summary of effects of metabolites of myclobutanil on aquatic organisms

Test species	**Test substance**	**Time-scale**	**Endpoints**	**References**
Oncorhynchus mykiss	myclobutanil butyric acid	96 h static	LC_{50} > 100 mg/L (nom)	Marino T. A. *et al.*, 2004a
Daphnia magna	myclobutanil butyric acid	48 h static	EC_{50} > 100 mg/L (nom)	Marino T. A. *et al.*, 2004b
Pseudokirchneriella subcapitata	myclobutanil butyric acid	96 h static	E_bC_{50} = 56.2 mg/L E_rC_{50} = 69.2 mg/L (mm)	Hancock G. A. *et al.*, 2004
Lemna gibba	myclobutanil butyric acid	7 d static	EC_{50} > 105 mg/L (mm)	Hancock G. A. *et al.*, 2004

Table B.9.2.15-3 : Summary of effects of the formulations Systhane 20 EW and GF-1317 on aquatic organisms

Test species	**Test substance**	**Time-scale**	**Endpoints**	**References**
Oncorhynchus mykiss	Systhane 20 EW	96 h static	LC_{50} = 10.3 mg Systhane 20 EW/L (**2.04 mg a.s./L**) (mm)	Naudin S., 1997
Daphnia magna	Systhane 20 EW	48 h static	EC_{50} = 7.1 mg Systhane 20 EW/L (**1.41 mg a.s./L**) (mm)	Naudin S., 1997
Daphnia magna	GF-1317	21 d semi-static	NOEC = 1.3 mg GF-1317/L (**0.27 mg a.s./L**) (nom)	Cafarella M. A., 2004
Pseudokirch-neriella subcapitata	Systhane 20 EW	96 h static	E_bC_{50} = 8.6 mg Systhane 20 EW/L (1.70 mg a.s./L) E_rC_{50} > 5.0 mg Systhane 20 EW/L (**0.99 mg a.s./L**) (mm)	Naudin S., 1997

Systhane 20 EW : formulation containing 19.8 % myclobutanil (batch n°: DK-2102-A)
GF-1317 : formulation containing 20.6 % myclobutanil (batch n°: E1743-16)

B.9.2.16 Exposure and risk assessment for aquatic organisms (Annex IIIA 10.2)

The intended uses of myclobutanil are :

- grapes : 4 applications of maximum 0.048 kg a.s./ha
- apples : 4 applications of maximum 0.090 kg a.s./ha

In the update of the section on fate and behaviour the PEC_{SW} and PEC_{SED} values were calculated according to FOCUS step 1, step 2, step 3 and step 4.

A rough estimation based on the worst case PEC_{SW} values step 1 (max PEC_{SW} = 47.9 µg a.s./L for grapes and max PEC_{SW} = 106.1 µg a.s./L for apples) and step 2 (max PEC_{SW} NE = 8.7 µg a.s./L and max PEC_{SW} SE = 10.9 µg a.s./L for grapes, max PEC_{SW} NE = 27.2 µg a.s./L and max PEC_{SW} SE = 35.4 µg a.s./L for apples) and the endpoints from the laboratory studies shows that no acceptable risk to aquatic organisms can be demonstrated. Therefore the aquatic risk assessment is conducted with FOCUS step 3 for grapes and step 4 for apples.

Table B.9.2.16-1 : Summary of PEC_{SW} and PEC_{SED} values for myclobutanil (Step 3 minimum default no-spray zones) following use of Systhane 20EW on vines (late application - worst case for spray drift)

Concentration	**D6 d (3.5 m)**	**R1 p (6 m)**	**R1 s (4 m)**	**R2 s (4 m)**	**R3 s (4 m)**	**R4 s (4 m)**
(µg a.s./L)						
Max. PEC_{SW}	0.873	0.092	1.135	0.662	0.699	1.536
TWA PEC_{SW} 21 d	0.526	0.081	0.052	0.025	0.025	0.082
(µg a.s./kg)						
Max. PEC_{SED}	2.738	0.560	0.642	0.471	0.268	1.168
TWA PEC_{SED} 21 d	2.451	0.559	0.271	0.296	0.127	0.511

Table B.9.2.16-2 : Summary of PEC_{SW} and PEC_{SED} values for myclobutanil (Step 4 refined no-spray zones) following use of Systhane 20EW on apples (early application - worst case for spray drift)

Concentration	**D3 d (12 m)**	**D4 p (6 m)**	**D4 s (14 m)**	**D5 p (6 m)**	**D5 s (14 m)**
(µg a.s./L)					
Max. PEC_{SW}	2.135	1.588	1.841	1.425	2.005
TWA PEC_{SW} 21 d	0.256	1.552	0.892	1.335	0.302
(µg a.s./kg)					
Max. PEC_{SED}	1.806	13.79	3.318	12.570	1.488
TWA PEC_{SED} 21 d	1.057	13.788	3.159	12.566	1.344

Concentration	**R1 p (6 m)**	**R1 s (14 m)**	**R2 s (14 m)**	**R3 s (14 m)**	**R4 s (14 m)**
(µg a.s./L)					
Max. PEC_{SW}	1.021	2.036	1.901	2.003	1.909
TWA PEC_{SW} 21 d	0.920	0.090	0.089	0.116	0.184
(µg a.s./kg)					
Max. PEC_{SED}	5.624	1.146	1.271	0.794	1.523
TWA PEC_{SED} 21 d	5.620	0.454	0.626	0.432	0.717

Risk assessment of myclobutanil for aquatic organisms :

Acute TER calculations and the risk to sediment dwelling organisms were performed with the following initial FOCUS PEC_{SW} values to cover the range of PEC_{SW} values for different scenarios :

Grapes : Max PEC_{SW} (scenario R 4 s, 4 m buffer zone) = 1.536 µg a.s./L
Max PEC_{SW} (scenario R 1 p, 6 m buffer zone) = 0.092 µg a.s./L

Apples : Max PEC_{SW} (scenario D 3 d, 12 m buffer zone) = 2.135 µg a.s./L
Max PEC_{SW} (scenario R 1 p, 6 m buffer zone) = 1.021 µg a.s./L

Table B.9.2.16-3 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to myclobutanil for the use in grapes (4 x 0.048 kg a.s./ha)

Test substance	**Scena-rio**	**Water body type**	**Test species**	**Time-scale**	**End-point (mg a.s./L)**	**Buffer-zone**	**Max PEC_{SW}, (µg a.s./L)**	**TER**	**Annex VI Trigger value**
myclobutanil	R 4	stream	*Oncorhynchus mykiss*	96 h static	2.0	4 m	1.536	1302	100
	R 1	pond				6 m	0.092	21739	100
Systhane 20 EW	R 4	stream	*Oncorhynchus mykiss*	96 h static	2.04	4 m	1.536	1328	100
	R 1	pond				6 m	0.092	22174	100
myclobutanil	R 4	stream	*Mysidopsis bahia*	96 h flow-through	0.24	4 m	1.536	156	100
	R 1	pond				6 m	0.092	2609	100
Systhane 20 EW	R 4	stream	*Daphnia magna*	48 h static	1.41	4 m	1.536	918	100
	R 1	pond				6 m	0.092	15326	100
myclobutanil	R 4	stream	*Pseudokirch-neriella subcapitata*	120 h static	1.1	4 m	1.536	716	10
	R 1	pond				6 m	0.092	11957	10
Systhane 20 EW	R 4	stream	*Pseudokirch-neriella subcapitata*	96 h static	>0.99	4 m	1.536	>645	10
	R 1	pond				6 m	0.092	>10761	10
myclobutanil	R 4	stream	*Chironomus riparius*	30 d static	4.98	4 m	1.536	3242	10
	R 1	pond				6 m	0.092	54130	10

Systhane 20 EW : formulation containing 19.8 % myclobutanil (batch n°: DK-2102-A)

Table B.9.2.16-4 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to myclobutanil for the use in apples (4 x 0.090 kg a.s./ha)

Test substance	Scena-rio	Water body type	Test species	Time-scale	End-point (mg a.s./L)	Buffer-zone	Max PEC_{SW}, (µg a.s./L)	TER	Annex VI Trigger value
myclobutanil	D 3	ditch	*Oncorhynchus mykiss*	96 h static	2.0	12 m	2.135	937	100
	R 1	pond				6 m	1.021	1959	100
Systhane 20 EW	D 3	ditch	*Oncorhynchus mykiss*	96 h static	2.04	12 m	2.135	956	100
	R 1	pond				6 m	1.021	1998	100
myclobutanil	D 3	ditch	*Mysidopsis bahia*	96 h flow-through	0.24	12 m	2.135	112	100
	R 1	pond				6 m	1.021	235	100
Systhane 20 EW	D 3	ditch	*Daphnia magna*	48 h static	1.41	12 m	2.135	660	100
	R 1	pond				6 m	1.021	1381	100
myclobutanil	D 3	ditch	*Pseudokirch-neriella subcapitata*	120 h static	1.1	12 m	2.135	515	10
	R 1	pond				6 m	1.021	1077	10
Systhane 20 EW	D 3	ditch	*Pseudokirch-neriella subcapitata*	96 h static	>0.99	12 m	2.135	>464	10
	R 1	pond				6 m	1.021	>970	10
myclobutanil	D 3	ditch	*Chironomus riparius*	30 d static	4.98	12 m	2.135	2333	10
	R 1	pond				6 m	1.021	4878	10

Systhane 20 EW : formulation containing 19.8 % myclobutanil (batch n°: DK-2102-A)

Chronic TER calculations were performed with the following FOCUS PEC_{SW} values :

Grapes : TWA PEC_{SW} (scenario D 6 d, 3.5 m buffer zone) = 0.526 µg a.s./L
TWA PEC_{SW} (scenario R 2 s, 4 m buffer zone) = 0.025 µg a.s./L

Apples : TWA PEC_{SW} (scenario D 4 p, 6 m buffer zone) = 1.552 µg a.s./L
TWA PEC_{SW} (scenario R 1 s, 14 m buffer zone) = 0.090 µg a.s./L

Table B.9.2.16-5 : Chronic Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to myclobutanil for the use in grapes (4 x 0.048 kg a.s./ha)

Test substance	Scena-rio	Water body type	Test species	Time-scale	End-point (mg a.s./L)	Buffer-zone	TWA PEC_{SW}, (µg a.s./L)	TER	Annex VI Trigger value
myclobutanil	D 6	ditch	*Oncorhynchus mykiss*	21 d flow-through	0.2	3.5 m	0.526	380	10
	R 2	stream				4 m	0.025	8000	10
myclobutanil	D 6	ditch	*Daphnia magna*	21 d semi-static	1.0	3.5 m	0.526	1901	10
	R 2	stream				4 m	0.025	40000	10
GF-1317	D 6	ditch	*Daphnia magna*	21 d semi-static	0.27	3.5 m	0.526	513	10
	R 2	stream				4 m	0.025	10800	10

GF-1317 : formulation containing 20.6 % myclobutanil (batch n°: E1743-16)

Table B.9.2.16-6 : Chronic Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to myclobutanil for the use in apples (4 x 0.090 kg a.s./ha)

Test substance	Scena-rio	Water body type	Test species	Time-scale	End-point (mg a.s./L)	Buffer-zone	TWA PEC_{SW}, (µg a.s./L)	TER	Annex VI Trigger value
myclobutanil	D 4	pond	*Oncorhynchus mykiss*	21 d flow-through	0.2	6 m	1.552	129	10
	R 1	stream				14 m	0.090	2222	10
myclobutanil	D 4	pond	*Daphnia magna*	21 d semi-static	1.0	6 m	1.552	644	10
	R 1	stream				14 m	0.090	11111	10
GF-1317	D 4	pond	*Daphnia magna*	21 d semi-static	0.27	6 m	1.552	174	10
	R 1	stream				14 m	0.090	3000	10

GF-1317 : formulation containing 20.6 % myclobutanil (batch n°: E1743-16)

The acute risk in grapes is acceptable with a no-spray zone of 4 m based on the worst case scenario R 4 stream.
The chronic risk in grapes is acceptable with a no-spray zone of 3.5 m based on the worst case scenario D 6 ditch.
The acute risk in apples is acceptable with a no-spray zone of 12 m based on the worst case scenario D 3 ditch.
The chronic risk in apples is acceptable with a no-spray zone of 6 m based on the worst case scenario D 4 pond.

Risk assessment of the metabolites of myclobutanil for aquatic organisms :

The metabolite myclobutanil butyric acid is less toxic to fish, aquatic invertebrates, algae and aquatic plants than the active substance myclobutanil. Therefore, myclobutanil butyric acid reveals no concern.

In conclusion, the risk of myclobutanil to aquatic organisms is acceptable with a no-spray zone of 4 m for the intended use in grapes and with a no-spray zone of 12 m for the intended use in apples.

B.9.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3)

The ecotoxicologically relevant endpoints for mammals are derived from the section on mammalian toxicology. To assess the long term risk to mammals the endpoint of the two-generation reproduction study in rat is used.

Table B.9.3-1 : Summary of effects of myclobutanil on mammals

Test species	Test system	Results	References
Rat	acute oral	**LD_{50} = 1600 mg a.s./kg b.w. (male rat)**	Krzywicki and Morrisson, 1984
Rat	2-generation reproduction	**NOEL = 16 mg a.s./kg b.w./day**	Costlow R.D. and Harris J.C., 1985

First tier risk assessment for mammals :

The risk assessment for mammals is based on the new Guidance Document for birds and mammals Under Council Directive 91/414/EEC of November 2002. As a worst case it was assumed that the mammals obtained 100 % of their diet in the treated area.

Table B.9.3-2 : Estimated oral uptake of myclobutanil by mammals and first tier Toxicity Exposure Ratio's (TER's) for use in grapes (4 x 0.048 kg a.s./ha) and in apples (4 x 0.090 kg a.s./ha)

Application rate	Mammal type	Time-scale	FIR/b.w.	RUD	MAF	f_{twa}	ETE	TER	Annex VI trigger value
4 x 0.048 kg a.s./ha in grapes	small herbivorous mammal	acute	1.39	85	1.6	-	9.07	176	10
		long-term	1.39	46	1.9	0.53	3.09	5.18	5
4 x 0.090 kg a.s./ha in apples	small herbivorous mammal	acute	1.39	85	1.6	-	17.0	94	10
		long-term	1.39	46	1.9	0.53	5.79	2.76	5

ETE is expressed in mg a.s./kg b.w./day

The risk of myclobutanil for small herbivorous mammals in grapes at 4 applications of maximum 0.048 kg a.s./ha is acceptable for acute and long-term exposure.
The risk of myclobutanil for small herbivorous mammals in apples at 4 applications of maximum 0.090 kg a.s./ha is acceptable for acute exposure but is not acceptable for long-term exposure. The long-term risk for small herbivorous mammals in apples has to be refined.

Refinement of the long-term risk to small herbivorous mammals :

1. *Application during flowering in vines (4 x 0.048 kg a.s./ha) and during foliage development (4 x 0.090 kg a.s./ha) in apples :*

The calculation of RUD in first tier approach is based on a crop interception factor of 40 %, giving the value of 46. Since the application of myclobutanil in both grapes and apples is intended during fruit development this interception factor can be refined. According to the FOCUS groundwater scenarios the interception factor for

foliage development in apples is 70 % and the interception factor for flowering in vines is also 70 %. The RUD factor is reduced to 22.8, this is 30 % of 76.

Table B.9.3-3 : Estimated oral uptake of myclobutanil by mammals and higher tier Toxicity Exposure Ratio's (TER's) for use in grapes (4 x 0.048 kg a.s./ha) and in apples (4 x 0.090 kg a.s./ha)

Application rate	**Mammal type**	**Time-scale**	**FIR/b.w.**	**RUD**	**MAF**	**f_{twa}**	**ETE**	**TER**	**Annex VI trigger value**
4 x 0.048 kg a.s./ha in grapes	small herbivorous mammal	long-term	1.39	22.8	1.9	0.53	1.53	10.4	5
4 x 0.090 kg a.s./ha in apples	small herbivorous mammal	long-term	1.39	22.8	1.9	0.53	2.87	5.57	5

ETE is expressed in mg a.s./kg b.w./day

The long-term risk of myclobutanil for small herbivorous mammals in grapes at 4 applications of maximum 0.048 kg a.s./ha and in apples at 4 applications of maximum 0.090 kg a.s./ha is acceptable with the refined RUD value of 22.8.

2. *Application during flowering (2 x 0.090 kg a.s./ha) and during foliage development (2 x 0.090 kg a.s./ha) in apples :*

For the use of myclobutanil in apples, applications can be made at an earlier stage than foliar development, that being at flowering. The notifier proposes 2 applications at flowering and 2 applications at foliage development. According to the FOCUS groundwater scenarios the interception factor for flowering is 65 % and for foliage development is 70 %. The corresponding RUD factors are reduced to 26.6 and 22.8, this is 35 % respectively 30 % of 76.

Table B.9.3-4 : Estimated oral uptake of myclobutanil by mammals and higher tier Toxicity Exposure Ratio's (TER's) for use in apples (4 x 0.090 kg a.s./ha)

Application rate	**Mammal type**	**Time -scale**	**FIR/b.w.**	**RUD**	**MAF**	**f_{twa}**	**ETE**	**TER**	**Annex VI trigger value**
2 x 0.090 kg a.s./ha during flowering	small herbivorous mammal	long-term	1.39	26.6	1.5	0.53	2.65	6.04	5
2 x 0.090 kg a.s./ha during foliage development	small herbivorous mammal	long-term	1.39	22.8	1.5	0.53	2.27	7.05	5

ETE is expressed in mg a.s./kg b.w./day

The long-term risk of myclobutanil for small herbivorous mammals in apples at 2 applications during flowering and at 2 applications during foliage development is acceptable with the refined RUD values of 26.6 respectively 22.8.

Risk of myclobutanil to earthworm-eating and fish-eating mammals :

Since the log P_{OW} of myclobutanil is around 3, no risk assessment on bioaccumulation for earthworm-eating mammals or fish-eating mammals is needed according to the Guidance Document for birds and mammals. The notifier provided an additional study on the bioaccumulation in earthworms, showing a low BCF value of 0.46 – 0.47.

In conclusion, the risk of myclobutanil to mammals is acceptable for the intended use in grapes and in apples.

B.9.4 Effects on bees (Annex IIA 8.3.1; Annex IIIA 10.4)

B.9.4.8 Exposure and risk assessment for bees (Annex IIIA 10.4)

Table B.9.4.8-1 : Summary of effects of the formulation Systhane 20 EW on bees

Test species	Test system	Endpoints	References
Apis mellifera	acute oral (72 h)	**LD_{50} oral > 171 µg Systhane 20 EW/bee** (33.9 µg a.s./bee)	Candolfi M.P., 1996
	acute contact (72 h)	**LD_{50} contact > 200 µg Systhane 20 EW/bee** (39.6 µg a.s./bee)	Candolfi M.P., 1996

Systhane 20 EW : formulation containing 19.8 % myclobutanil (batch n°: DK-2102-A)

First tier risk assessment for bees :

Table B.9.4.8-2 : Hazard quotients for bees exposed to myclobutanil for use in grapes (4 x 0.048 kg a.s./ha) and in apples (4 x 0.090 kg a.s./ha)

Application rate	Crop	Route	Hazard quotient	Annex VI Trigger
Laboratory tests				
240 g Systhane 20 EW/ha	grapes	oral	< 1.4	50
		contact	< 1.2	50
450 g Systhane 20 EW/ha	apples	oral	< 2.6	50
		contact	< 2.3	50

The hazard quotients for use in grapes were calculated with the maximum single application rate of 0.048 kg a.s./ha equivalent with 240 g Systhane 20 EW/ha.
The hazard quotients for use in apples were calculated with the maximum single application rate of 0.090 kg a.s./ha equivalent with 450 g Systhane 20 EW/ha.

In conclusion, the risk of myclobutanil and the formulation Systhane 20 EW to bees is acceptable for the intended use in grapes and in apples.

B.9.5 Effects on other arthropod species (Annex IIA 8.3.2; Annex IIIA 10.5)

B.9.5.1 Effects of the active substance on non-target terrestrial arthropods (Annex IIA 8.3.2)

Studies were performed with appropriate formulations.

B.9.5.2 Effects of the formulations on non-target terrestrial arthropods (laboratory, semi-field tests) (Annex IIIA 10.5.1)

Systhane® 20EW: Laboratory Contact Toxicity Test with the Predacious Mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) based on the IOBC Approved Method of Overmeer (1988). (Candolfi M., 1996).

Guidelines :
Overmeer, W.P.J. (1988). Laboratory method for testing side-effects of pesticides on the predacious mites *Typhlodromus pyri* and *Amblyseius potentillae* (Acari: Phytoseiidae). In: IOBC Working Group Pesticides and Beneficial Organisms, Guidelines for Testing the Effects of Pesticides on Beneficials: Short Description of Test Methods. IOBC/WPRS Bulletin XI/4:65-69
GLP :
Yes
Material and Methods :
Test substance : Systhane 20 EW, formulation containing 19.8 % myclobutanil, batch n°: DK-2102-A
Test species : Typhlodromus pyri (predacious mite), protonymphs
Number of organisms : 5 replicates per treatment each containing 20 protonymphs
Type of test : laboratory test
Applied and measured concentrations :
control (water); positive control (ethyl parathion); treatment at 181.8 g Systhane 20 EW in 200 L water/ha (equivalent to 36 g a.s./ha)
Exposure route :
The glass plates were sprayed and left to air dry. Twenty protonymphs of *Typhlodromus pyri* were added to each test unit. A minute amount of pollen was provided as a food supply.
Test conditions :
temperature : 24.0 – 26.0 °C
relative humidity : 70 – 80 %
light intensity : 1241 lux
photoperiod : 16 hours light, 8 hours dark cycle
Findings :

Table B.9.5.2-1 : Effects of the formulation Systhane 20 EW on *Typhlodromus pyri* (laboratory test)

Evaluation criteria	Control	Positive Control	Systhane 20 EW
Mortality after 7 days (%)	1.0 ± 2.2	80.0 ± 20.3	51.0 ± 13.9*
Corrected mortality (%)	-	79.8	50.5
Mean number of eggs per female per day	1.07 ± 0.53	0.15 ± 0.15	0.35 ± 0.12*
Reproduction relative to control (%)	-	- 86.0	- 67.3

* statistically significant different from the control

The percent cumulative mortality in the treatment group was statistically significantly different from the control. The females treated with Systhane 20 EW produced significantly less eggs per female per day when compared to the control.
Conclusions :

The study is acceptable.
Systhane® 20 EW: Laboratory Contact Toxicity Test with the Seven-Spotted Lady Beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), Based on the Method of Pinsdorf (1989). (Candolfi M.P., 1996).

Guidelines :
BBA Guideline, Section VI, 23-2.1.5, 1989
GLP :
Yes
Material and Methods :
Test substance : Systhane 20 EW, formulation containing 19.8 % myclobutanil, batch n°: DK-2102-A
Test species : Coccinella septempunctata, second instar larvae (3 days old)
Number of organisms :
Exposure phase : 5 replicates per treatment each containing 10 lady beetle larvae
Reproduction phase : the lady beetles from each treatment were pooled to form groups of ≤ 25 beetles with a sex ratio of approximately 1:1
Type of test : laboratory test
Applied and measured concentrations :
control (water); positive control (pyrazophos); treatment at 181.8 g Systhane 20 EW in 200 L water/ha (equivalent to 36 g a.s./ha)
Exposure route :
The lady beetle larvae were exposed to treated glass plates until they reached the adult stage (approximately 2 weeks). Thereafter the surviving beetles were pooled with their respective treatment groups. One batch of beetles (≤ 25) from each treatment group with the sex ratio of approximately 1:1 was impartially selected and placed into separate test units (cages) used to assess the reproductive performance during a period of 5 weeks. Pea aphids (*Acyrthosiphon pisum*) and cereal aphids (*Rhopalosiphum padi*) were supplied as food.
Test conditions :
temperature : 22.0 – 25.0 °C
relative humidity : 62 - 80 %
light intensity : 1998 lux
photoperiod : 16 hours light, 8 hours dark cycle
Findings :

Table B.9.5.2-2 : Effects of the formulation Systhane 20 EW on *Coccinella septempunctata* (laboratory test)

Evaluation criteria	**Control**	**Positive Control**	**Systhane 20 EW**
Mean cumulative mortality after 16 days (%)	16.0 ± 5.5	100 ± 0.0	26.0 ± 16.7
Corrected mortality (%)	-	100	11.9
Mean number of eggs per female per day	6.46 ± 4.95	-	4.07 ± 4.04
Hatching rate (%)	76.00 ± 26.28	-	60.32 ± 27.00
Number of viable eggs per female	108.74	-	52.39
Reproduction relative to control (%)	-	-	- 50.02

F = ((number of eggs per female) x hatching rate in %)/100
F_t = average number of fertile eggs per female in the treatment group = 2.454
F_c = average number of fertile eggs per female in the control group = 4.909

Conclusions :
The study is acceptable.

Systhane® 20EW: Laboratory Contact Toxicity Test with Spiders, *Pardosa* sp. (Araneae: Lycosidae) based on the BBA Method of Wehling and Heimbach (1994). (Candolfi M., 1996).

Guidelines :
BBA VI 23-2.1.9 (Wehling and Heimbach, 1994)
GLP :
Yes
Material and Methods :
Test substance : Systhane 20 EW, formulation containing 19.8 % myclobutanil, batch n°: DK-2102-A
Test species : Pardosa sp., collection end of February, no indication of age
Number of organisms : 20 replicates per treatment (10 replicates with each one male spider and 10 replicates each with one female spider)
Type of test : laboratory test
Applied and measured concentrations :
control (water); positive control (lambda cyhalothrin); treatment at 227.3 g Systhane 20 EW in 500 L water/ha (equivalent to 45 g a.s./ha)
Exposure route :
The spiders were held individually in small plastic containers filled with 125 g pure quartz sand. The test units were sprayed and food (2 *Delia antiqua* flies) was added to each test unit. During testing the sand was remoistured every day to approximately 70 % of the maximum water holding capacity. Mortality, behaviour and feed consumption were monitored for 14 days.
Test conditions :
temperature : 18.0 – 21.5 °C
relative humidity : 67 – 84 %
light intensity : 1194 lux
photoperiod : 16 hours light, 8 hours dark cycle
Findings :

Table B.9.5.2-3 : Effects of the formulation Systhane 20 EW on *Pardosa* sp. (laboratory test)

Evaluation criteria	**Control**	**Positive Control**	**Systhane 20 EW**
Cumulative mortality (%)			
- males	10.0 ± 31.6	100.0 ± 0	20.0 ± 42.2
- females	10.0 ± 31.6	100.0 ± 0	10.0 ± 31.6
Corrected mortality (%)	-	100	5.6
Number of flies consumed per spider per day			
- males	0.2 ± 0.05	-	0.1 ± 0.1
- females	0.3 ± 0.1	-	0.3 ± 0.2
- males + females	0.3 ± 0.1	-	0.2 ± 0.2
Food consumption relative to control (%)	-	-	- 33.3

No significant difference in mortality, behaviour or average feeding rate was observed between the treatment and the control groups.
Conclusions :
The study is acceptable.

Systhane 20EW: Laboratory Toxicity Test with the Parasitic Wasp, *Aphidius rhopalosiphi* (Hymnepotera: Braconidae) Based on the IOBC Approved Method of Polgar (1988). (Candolfi M.P., 1996).

Guidelines :
Polgar, L. 1988. Guideline for testing the effect of pesticides on *Aphidius rhopalosiphi* Hal. Hym., Aphidiidae: Laboratory contact tests: 1-on adults, 2-on aphid mummies, semi-field tests on adults. In: IOBC/WPRS Bulletin XI/4; Meeting of the Working Group "Pesticides and Beneficial Organisms". pp: 29-34.
GLP :
Yes
Material and Methods :
Test substance : Systhane 20 EW, formulation containing 19.8 % myclobutanil, batch n°: DK-2102-A
Test species : Aphidius rhopalosiphi, adult wasps, less than 48 hours old
Number of organisms :
Exposure phase : 3 replicates per treatment each containing 10 female wasps
Reproduction phase : all surviving healthy females per treatment were housed individually
Type of test : extended laboratory test
Applied and measured concentrations :
control (water); positive control (dimethoate); treatment at 181.8 g Systhane 20 EW in 200 L water/ha (equivalent to 36 g a.s./ha)
Exposure route :
Adult wasps were exposed to treated glass surfaces for 24 hours. All surviving healthy females were individually transferred to potted barley plants. These barley plants were infested with at least 40 II/II-instar nymphs of the cereal aphid *Rhopalosiphum padi*. The period of parasitisation lasted for 24 hours. The number of mummies (parasitised aphids) that had developed was recorded 10 ± 2 days after parasitisation.
Test conditions :
temperature : 18.5 – 20.5 °C
relative humidity : 70 – 83 %
light intensity : 979 lux (exposure phase), 2496 lux (reproduction phase)
photoperiod : 16 hours light, 8 hours dark cycle
Findings :

Table B.9.5.2-4 : Effects of the formulation Systhane 20 EW on *Aphidius rhopalosiphi* (extended laboratory test)

Evaluation criteria	**Control**	**Positive Control**	**Systhane 20 EW**
Mortality after 48 hours (%)	0 ± 0	93.3 ±11.5	0 ± 0
Corrected mortality (%)	-	93.3	0.00
Mean number of mummies per female	32.3 ± 14.5	-	18.4 ± 15.6*
Reproduction relative to control (%)	-	-	- 43.0

* statistically significant different from the control

The females exposed to the formulation parasitised significantly less aphids when compared to the control.
Conclusions :
The study is acceptable.

Extended Laboratory Test to Evaluate the Side Effects of Systhane® 20EW Applied to Plants on Adult *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) Based on Mead-Briggs (1994). (Candolfi M.P., 1996).

Guidelines :
Mead-Briggs, M. 1994. An Extended Laboratory Test to Evaluate the Side-effects of Pesticides Applied to Plant Material on Adults of the Aphid Parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). (personal written communication to the author)
GLP :
Yes
Material and Methods :
Test substance : Systhane 20 EW, formulation containing 19.8 % myclobutanil, batch n°: DK-2102-A
Test species : Aphidius rhopalosiphi, adult wasps, less than 48 hours old
Number of organisms :
Exposure phase : 6 replicates per treatment each containing 5 female wasps
Reproduction phase : maximum of 6 replicates per treatment containing 3 female wasps, depending on survival
Type of test : extended laboratory test
Applied and measured concentrations :
control (water); positive control (dimethoate); treatment at the maximum recommended field application rate of 90 g a.s. in 200 L water/ha
Exposure route :
The barley plants for the exposure phase were pre-treated with a fructose solution (25 % w/v in water) and thereafter sprayed with the test substance. The sugar provided both food and a foraging stimulus for the wasps. The adult wasps were exposed for 48 hours to the treated plants which were enclosed in untreated acrylic cylinders. The plants for the fecundity assessments were infested with adult cereal aphids *Rhopalosiphum padi* and enclosed in cylinders. The surviving wasps were left to parasitise the aphid-infested plants and were removed after 24 hours. Any aphid mummies which subsequently developed were counted 10 days later.
Test conditions :
temperature : 18.0 – 20.5 °C
relative humidity : 68 – 90 %
light intensity : 1120 lux (exposure phase), 2200 lux (reproduction phase)
photoperiod : 16 hours light, 8 hours dark cycle
Findings :

Table B.9.2.5-5 : Effects of the formulation Systhane 20 EW on *Aphidius rhopalosiphi* (extended lab test)

Evaluation criteria	**Control**	**Positive Control**	**Systhane 20 EW**
Exposure phase			
Mortality after 48 hours (%)	3.3 ± 8.2	100.0 ± 0	43.3 ± 42.7*
Corrected mortality (%)	-	100.0	41.4
Affected after 48 hours (%)	0 ± 0	0 ± 0	16.7 ± 8.2*
Reproduction phase			
Mortality after 24 hours (%)	16.7 ± 8.2*	-	58.3 ± 25.3*
Mean number of mummies per female	30.3 ± 15.2	-	14.3 ± 12.8
Reproduction relative to control (%)	-	-	- 52.8

* statistically significant different from the control

Conclusions :
The study is acceptable.

An aged residue study to evaluate the effects of three rates of myclobutanil (a 20EW formulation) on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera Braconidae). (Davies N.A., 2003).

Guidelines :
A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera: Braconidae)". 2000. Mead-Briggs M. A., Brown K., Candolfi M. P., Coulson M. J. M., Miles M., Moll M., Nienstedt K., Schuld M., Ufer A. and McIndoe E.
GLP :
Yes
Material and Methods :
Test substance : Systhane 20 EW, formulation containing 211 g myclobutanil/L, batch n°: QC2388R301
Test species : Aphidius rhopalosiphi, adult wasps, 1 day old
Number of organisms :
Exposure phase : 6 replicates per treatment each containing 5 wasps
Reproduction phase : 15 female wasps were individually exposed to aphid infested plants
Type of test : extended laboratory test
Applied and measured concentrations :
control (water); positive control (dimethoate); treatment at application rates of 288, 780 and 1200 g a.s. in 400 L water/ha
Exposure route :
The plants for the exposure phase were pre-treated with a 10 % w/v fructose solution in water and thereafter sprayed with the test substance. The fructose acted as a food source and foraging stimulus for the wasps. The adult wasps were exposed for 48 hours to the treated plants which were enclosed within a clear plastic cylinder. As a measure of repellence of insects from the treated foliage, the number of wasps settled on the plants were counted. The plants for the fecundity assessments were infested with bird cherry-oat aphids *Rhopalosiphum padi* and were not sprayed with the test substance. The surviving wasps were left to parasitise the aphid-infested plants and were removed after 24 hours. The number of parasitised aphids were counted 11 days later. As there was no effect of test item equal to or greater than 25 % mortality of the wasps on the 0DAA plants, the bioassay on the 14DAA plants was not carried out.
Test conditions :
temperature : 20.5 – 23 °C (exposure phase), 16.9 – 26.1 °C (reproduction phase)
relative humidity : 50 – 75 %
light intensity : 993 lux (exposure phase), 8318 lux (reproduction phase)
photoperiod : 16 hours light, 8 hours dark cycle
Findings :

Table B.9.2.5-6 : Effects of the formulation Systhane 20 EW (expressed as active substance) on *Aphidius rhopalosiphi* (extended lab test)

Evaluation criteria	**Control**	**Positive control**	**Systhane 20 EW**		
			288 g a.s./ha	**780 g a.s./ha**	**1200 g a.s./ha**
Mortality after 48 hours (%)	0.00	90.00*	0.00	0.00	6.67
Corrected mortality (%)	-	90.0	0.00	0.00	6.67
Mean wasp settlement on the plant	44.00	26.00	24.67	31.33	37.33
Mean number of mummies produced per wasp (24 h)	18.20	-	20.07	16.27	18.60
Reproduction relative to control (%)	-	-	110	- 10.6	102

* statistically significant different from the control

No visible abnormalities or sub-lethal effects were seen in this study. In terms of repellence there was no significant difference in the setting of wasps on the Systhane 20 EW treated foliage at any of the rates tested.
Conclusions :
The study is acceptable.

An aged residue study to evaluate the effects of three rates of myclobutanil (a 20 EW formulation) on the green lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae). (Riches M.N., 2003).

Guidelines :
Vogt H., Bigler F., Brown K., Candolfi M. P., Kemmeter F., Kühner Ch., Moll M., Travis A., Ufer A., Viñuela E., Waldburger M. and Waltersdorfer A. "Laboratory method to test effects of plant protection products on larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae)", 2000.
GLP :
Yes
Material and Methods :
Test substance : Systhane 20 EW, formulation containing 211 g myclobutanil/L, batch n°: QC2388R301
Test species : Chrysoperla carnea, green lacewing, 2-3 days old larvae
Number of organisms : 40 replicates per treatment with individually housed larvae
Type of test : extended laboratory test
Applied and measured concentrations :
control (water); positive control (dimethoate); treatment at actual application rates of 307, 766 and 1380 g a.s. in 200 L water/ha
Exposure route :
Two fully expanded leaves of the dwarf French bean *Phaseolus vulgaris* were sprayed with the test substance. The larvae were exposed to the leaves and kept individually in acrylic tubes. One to seven days after the larvae had pupated they were transferred to plastic Petri dishes until adult emergence. For the control and test item treatments only, the emerging adults were placed in oviposition chambers. Up to 20 adults from each treatment were placed in each chamber, with up to two chambers per treatment. The number of male and female lacewings in each rearing chamber was recorded. The fecundity assessment began six days after the first eggs were visible. The percentage of eggs hatched was calculated. As the effect of the test treatments was less than 50 % mortality (Abbott corrected) on the 0DAA plants, the bioassay on the 14DAA plants was not carried out.
Test conditions :
temperature : 23.8 – 29.9 °C
relative humidity : 46.6 – 94.0 %
light intensity : 1324 lux
photoperiod : 16 hours light, 8 hours dark cycle
Findings :

Table B.9.2.5-7 : Effects of the formulation Systhane 20 EW (expressed as active substance) on *Chrysoperla carnea* (extended lab test)

Evaluation criteria	**Control**	**Positive control**	**Systhane 20 EW**		
			307 g a.s./ha	**766 g a.s./ha**	**1380 g a.s./ha**
Corrected pre-imaginal mortality (%)	0.00	65.71*	11.43	28.57*	40.00*
Mean number of eggs per female per day	19.58	-	23.54	24.63	20.60
Mean hatching rate (%)	95.96	-	94.75	96.37	94.79
Reproduction relative to control (%)	-	-	119	126	104

* statistically significant different from the control

F = ((number of eggs per female) x hatching rate in %)/100
F_t (307 g a.s./ha) = average number of fertile eggs per female in the treatment group = 22.30
F_t (766 g a.s./ha) = average number of fertile eggs per female in the treatment group = 23.74
F_t (1380 g a.s./ha) = average number of fertile eggs per female in the treatment group = 19.53
F_c = average number of fertile eggs per female in the control group = 18.78

In terms of mortality Systhane 20 EW caused a significant increase in mortality in the lacewings at the rates 766 and 1380 g a.s./ha. The mortalities at these rates (28.57 and 40.00 % respectively) did not exceed the 50 % trigger value given in ESCORT 2 (Candolfi *et al*., 2000). Some lacewing adults emerged deformed but these were not included in the reproduction phase of the study. None of the rates of Systhane 20 EW tested (307, 766 and 1380 g a.s./ha) had treatment-related effects on the reproductive performance of surviving adult lacewings.
Conclusions :
The study is acceptable.

Systhane® 20EW: Semi-Field Toxicity Test on Hops with the Parasitic Wasp, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae). Nienstadt, K.M. (1999).

Guidelines :
Mead-Briggs, M. 1996. Semi-field bioassay to evaluating the effects of plant protection products, applied to a cereal crop, on the aphid-specific parasitoid *Aphidius rhopalosiphi.* Semi-field ring test method currently being ring-tested in Europe in a joint initiative of IOBC, BART EPPO and COMET
GLP :
Yes
Material and Methods :
Test substance : Systhane 20 EW, formulation containing 19.9 % myclobutanil, batch n°: ES-96018
Test species : Aphidius rhopalosiphi, adult wasps, less than 3 days old
Number of organisms : 40 wasps (20 males and 20 females) per hop plant
Type of test : semi-field test (hop plants)
Applied and measured concentrations :
control (water); positive control (dimethoate); treatment at 54 g a.s. in 1000 L water/ha and
treatment at 300 g a.s. in 1000 L water/ha, both applied 4 times at 10 ± 2 days interval
Exposure route :
Each hop plant (*Humulus lupulus*) was grown in individual pots and was used as a replicate. After the 1st and 4th application bioassays were performed. A net was used to enclose each hop plant together with two pots of untreated barley plants (approximately 10 cm height) located at two different heights respective to the hop plant (approximately 30 and 60 mm from the soil). Each barley plant pot was infested with approximately 150 *Rhopalosiphum padi* and was replaced daily during three consecutive days. The barley plants were transferred to an environmental chamber to assess the number of aphid mummies.
Approximately 1-2 hours before the 1st and 4th application, each hop plant was pre-treated with a fructose solution (25 % w/v in water) to attract the wasps to the plant surfaces. After application of the test substance and covering each hop plant by a net, 40 *Aphidius rhopalosiphi* (20 males and 20 females) were released into each test unit. Behaviour of *Aphidius rhopalosiphi* was recorded after releasing by observing if the wasps were on the recovering net or not.
Test conditions of the environmental chamber :
temperature : 19.0 – 21.5 °C (1st bioassay), 19.0 – 20.5 °C (2nd bioassay)
relative humidity : 57 – 98 % (1st bioassay), 67 – 83 % (2nd bioassay)
light intensity : 2000 – 2700 lux (1st bioassay), 1200 – 1900 lux (2nd bioassay)
photoperiod : 16 hours light, 8 hours dark cycle

Findings :

Table B.9.2.5-8 : Effects of the formulation Systhane 20 EW (expressed as active substance) on *Aphidius rhopalosiphi* (semi-field test)

Evaluation criteria	**Control**	**54 g a.s./ha**	**300 g a.s./ha**	**Positive control**
Total number of parasitised aphid mummies				
Bioassay 1	49.13 ± 17.51	31.38 ± 34.73	48.63 ± 25.67	0.25 ± 0.29
Bioassay 2	53.13 ± 31.29	54.00 ± 31.71	44.25 ± 7.80	0.0 ± 0.0
Number of Aphidius rhopalosiphi on the net after release				
Bioassay 1	19.0 ± 0.8	14.3 ± 2.6	21.5 ± 4.1	14.3 ± 1.7
Bioassay 2	13.8 ± 1.5	16.3 ± 2.1	16.8 ± 2.6	10.8 ± 4.1
Reduction in reproductive ability (%)				
Bioassay 1	-	36	1	-
Bioassay 2	-	- 1.6	16.7	-

Conclusions :
The study is acceptable.
Based on the reduction of reproductive ability of *Aphidius rhopalosiphi* produced by Systhane 20 EW applied on hop at the highest concentration, Systhane 20 EW was classified according to the IOBC scheme (Hassan, 1992) as “harmless” to *Aphidius rhopalosiphi* under semi-field conditions.

B.9.5.4 Summary of effects, exposure and risk assessment for non-target terrestrial arthropods

Table B.9.5.4-1 : Summary of effects and risk assessment of myclobutanil for non-target arthropods

Species	Life stage	Test substance, substrate and duration	Dose (g a.s./ha)[1, 2]	End point	effect[3]	Trigger value
Laboratory tests						
Typhlodromus pyri	proto-nymphs	Systhane 20 EW, glass plates, 14 d	36 g a.s./ha, initial	Corrected mortality Reproduction	50.5 % - 67.3 %	50 % 50 %
Coccinella septempunctata	larvae	Systhane 20 EW, glass plates, 2 + 5 weeks	36 g a.s./ha, initial	Corrected mortality Reproduction	11.9 % - 50.02 %	50 % 50 %
Pardosa sp.	-	Systhane 20 EW, sand, 14 d	45 g a.s./ha, initial	Corrected mortality Food consumption	5.6 % - 33.3 %	50 % 50 %
Extended laboratory tests						
Aphidius rhopalosiphi	adult females	Systhane 20 EW, barley plants, 2 + 12 d	36 g a.s./ha, initial	Corrected mortality Reproduction	0.00 % - 43.0 %	50 % 50 %
Aphidius rhopalosiphi	adult females	Systhane 20 EW, barley plants, 2 d + 10 d	90 g a.s./ha, initial	Corrected mortality Reproduction	41.4 % - 52.8 %	50 % 50 %
Aged residue tests						
Aphidius rhopalosiphi	adult females	Systhane 20 EW, barley plants, 2 d + 11 d	288 g a.s./ha, 0DAA	Corrected mortality Reproduction	0.00 % + 10.0 %	50 % 50 %
			780 g a.s./ha, 0DAA	Corrected mortality Reproduction	0.00 % - 10.6 %	50 % 50 %
			1200 g a.s./ha, 0DAA	Corrected mortality Reproduction	6.67 % + 2.0 %	50 % 50 %
Chrysoperla carnea	larvae	Systhane 20 EW, bean leaves	307 g a.s./ha, 0DAA	Corrected mortality Reproduction	11.43 % + 19.0 %	50 % 50 %
			766 g a.s./ha, 0DAA	Corrected mortality Reproduction	28.57 % + 26.0 %	50 % 50 %
			1380 g a.s./ha, 0DAA	Corrected mortality Reproduction	40.0 % + 4.0 %	50 % 50 %

[1] indicate whether initial or aged residues
[2] for preparations indicate whether dose is expressed in units of a.s. or preparation
[3] indicate if positive percentages relate to adverse effects or not
(for Reproduction parameter : negative % = adverse effect; positive % = no adverse effect)

Systhane 20 EW : formualation containing 19.8 % myclobutanil (batch n°: DK-2102-A)
formulation containing 211 g/L myclobutanil (batch n°: QC2388R301)

In the semi-field test with *Aphidius rhopalosiphi*, hop plants were sprayed at 54 g a.s./ha and at 300 g a.s./ha, both applied 4 times at 10 ± 2 days interval. Untreated barley plants, infested with aphids were placed next to the treated hop plants. The first bioassay was performed after the 1st treatment and the second bioassay was performed after the 4th treatment. The reduction in reproductive ability at the application rate of 54 g a.s./ha was 36 % (bioassay 1) and – 1.6 % (bioassay 2). The reduction in reproductive ability at the application rate of 300 g a.s./ha was 1 % (bioassay 1) and 16.7 % (bioassay 2). Systhane 20 EW has no effects on *Aphidius rhopalosiphi* up to 4 x 300 g a.s./ha.

In the field test with *Typhlodromus pyri*, an apple orchard in southern Germany was treated with 0.45 L Systhane 20 EW/ha (89 mL a.s./ha) and with 0.9 L Systhane 20 EW/ha (178 mL a.s./ha), both applied 9 times between the beginning of June and the beginning of September. No effects were observed for the predatory mites (eggs and adults) and for the spider mites (eggs and adults) up to 9 x 0.9 L Systhane 20 EW/ha (equivalent to 9 x 180 g a.s./ha).

Risk assessment for non-target arthropods :

The extended laboratory test with *Aphidius rhopalosiphi* shows that no effects on mortality or on reproduction will be observed at application rates up to 1200 g a.s./ha. Since the maximum application rate in grapes is 4 x 0.048 kg a.s./ha and in apples is 4 x 0.090 kg a.s./ha, the risk to *Aphidius rhopalosiphi* is covered by this extended laboratory test. The risk of myclobutanil to *Aphidius rhopalosiphi* is acceptable.

No effects on the beneficial capacity of *Typhlodromus pyri* have been observed in an orchard field study (GAP of 0.9 L Systhane 20 EW at 9 applications per season). This study covers the supported uses in grapes and apples. The risk of myclobutanil to *Typhlodromus pyri* is acceptable.

The extended laboratory test with *Chrysoperla carnea* shows that no effects on mortality or on reproduction will be observed at application rates up to 1380 g a.s./ha. This study covers the supported uses in grapes and apples. The risk of myclobutanil to *Chrysoperla carnea* is acceptable.

The laboratory test with *Pardosa* sp. shows that no effects on mortality or food consumption will be observed at the application rate of 227.3 g Systhane 20 EW/ha (equivalent to 45 g a.s./ha).

We consider the risk assessment complete as for Annex I inclusion is concerned. However, at MS level further testing on crop relevant species (*Orius*, *Anthocoris* …) with the relevant GAP is necessary.

In conclusion, the risk of myclobutanil to non-target arthropods is acceptable.

B.9.6 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.6.1)

B.9.6.6 Summary and risk assessment for earthworms (Annex IIIA 10.6.1)

Table B.9.6.6-1 : Summary of effects of myclobutanil, the metabolite myclobutanil butyric acid and the formulations Systhane 24 E and Systhane 20 EW on earthworms

Test species	Test system	Results	References
Lumbricus terrestris	acute toxicity	LC_{50} = 250 mg a.s./kg substrate	Swigert J.P., 1986
Eisenia fetida	acute toxicity	LC_{50} > 1000 mg myclobutanil butyric acid/kg substrate	Warbritton R., 2004
Eisenia fetida	acute toxicity	LC_{50} = 384 mg Systhane 24 E/kg substrate (99 mg a.s./kg substrate)	Candolfi M.P., 1996
Eisenia fetida	long-term toxicity	NOEC = 10.3 mg a.s./kg dry soil*	Nienstedt K.M., 1999

* the test was conducted with Systhane 20 EW but the results are expressed as active substance

Systhane 24 E : formulation containing 25.8 % myclobutanil (batch n°: DK-2195-A)
Systhane 20 EW : formulation containing 19.9 % myclobutanil (batch n°: ES-96018)

Systhane 24 E is a more concentrated formulation containing similar solvent compounds. Therefore, data from studies with Systhane 24 E are considered as a worst case and suitable for assessing the effects of Systhane 20 EW.

First tier risk assessment for earthworms :

Since the log P_{OW} of myclobutanil is higher than 2, the toxicity endpoints should be divided by 2.

Table B.9.6.6-2 : Summary of the corrected endpoints for the risk assessment for earthworms

Test species	Test system	Corrected endpoints
Lumbricus terrestris	acute toxicity	**$LC_{50,corr}$ = 125 mg a.s./kg substrate**
Eisenia fetida	acute toxicity	$LC_{50,corr}$ > 500 mg myclobutanil butyric acid /kg substrate
Eisenia fetida	acute toxicity	**$LC_{50,corr}$ = 192 mg Systhane 24 E/kg substrate** **(49.5 mg a.s./kg substrate)**
Eisenia fetida	long-term toxicity	**$NOEC_{corr}$ = 5.15 mg a.s./kg dry soil**

The PEC values in soil were obtained from the section on fate and behaviour :

Table B.9.6.6-3 : Summary of PEC_{soil} for myclobutanil

	Accumulation PEC_{soil} (mg a.s./kg soil)	
	vines	**apples**
PEC_{max} = concentration in soil immediately after last application at 5 cm soil depth	0.359	0.672
$PEC_{plateau}$ = plateau average PEC after repeated applications during several years at 5 cm soil depth	0.289	0.544

Table B.9.6.6-4 : First Tier Toxicity Exposure Ratio's (TER's) for earthworms exposed to myclobutanil for use in grapes (4 x 0.048 kg a.s./ha) and in apples (4 x 0.090 kg a.s./ha)

Application rate	**Crop**	**Test species**	**Test substance**	**Time-scale**	**PEC_{soil} (mg a.s./kg soil)**	**TER**	**Annex VI Trigger**
4 x 0.048 kg a.s./ha	grapes	*Lumbricus terrrestris*	myclobutanil	acute	0.359	348	10
		Eisenia fetida	Systhane 24 E	acute	0.359	138	10
		Eisenia fetida	Systhane 20 EW	long-term	0.289	17.8	5
4 x 0.090 kg a.s./ha	apples	*Lumbricus terrrestris*	myclobutanil	acute	0.672	186	10
		Eisenia fetida	Systhane 24 E	acute	0.672	73.7	10
		Eisenia fetida	Systhane 20 EW	long-term	0.544	9.47	5

Systhane 24 E : formulation containing 25.8 % myclobutanil (batch n°: DK-2195-A)
Systhane 20 EW : formulation containing 19.9 % myclobutanil (batch n°: ES-96018)

The acute and long-term risk of myclobutanil to earthworms in grapes at 4 applications of 0.048 kg a.s./ha is acceptable.
The acute and long-term risk of myclobutanil to earthworms in apples at 4 applications of 0.090 kg a.s./ha is acceptable.

First tier risk assessment of the metabolites of myclobutanil :

The study conducted with the soil metabolite myclobutanil butyric acid shows that the toxicity of this metabolite is much less than the active substance, at least by a factor of 4. The acute and long-term risk of the active substance to earthworms in grapes and apples is acceptable. Therefore, we consider that the acute and long-term risk of the metabolite is also acceptable.

In conclusion, the risk of myclobutanil to earthworms is acceptable.

B.9.7 Effects on other soil non-target macro-organisms (Annex IIIA 10.6.2)

Risk assessment for the litter bag studies :

According to the EPFES Guideline, the annual cumulative application should be made in 1 dose on bare soil or on soil with only little plant cover. The study of Galicia (2002) was conducted in grassland and no analytical measurement of the actual concentration of myclobutanil was performed. It is expected that the applied myclobutanil was intercepted by grass and therefore there is no indication that the straw in the litter bags was exposed to the correct dose of myclobutanil. The study of Mallet (2004) is acceptable because measurements of actual myclobutanil concentrations were performed.

According to the EPFES Guideline the litter bags should be placed at 5 cm soil depth and the top 10 cm of the test soil should contain the FOCUS PEC_{soil} plateau concentration at a soil depth of 20 cm. The annual cumulative dose is applied subsequently.

Table B.9.7-6 : Summary of PEC_{soil} for myclobutanil

	Accumulation PEC_{soil} (mg a.s./kg soil)	
	vines	**apples**
PEC_{max} = concentration in soil immediately after last application at 5 cm soil depth	0.359	0.672

PEC_{soil} after last application at 20 cm soil depth in vines = 0.090 mg a.s./kg soil
PEC_{soil} after last application at 20 cm soil depth in apples = 0.168 mg a.s./kg soil

In the study of Mallet (2004) it was shown that myclobutanil had no adverse effect on the rate of breakdown of straw litter in soil at mean concentrations of 0.1247 – 0.1460 mg a.s./kg soil. This concentration range covers the worst case PEC_{soil} of 0.168 mg a.s./kg soil.

In conclusion, the risk of myclobutanil to other non-target macro-organisms is acceptable.

B.9.8. Effects on soil non-target micro-organisms (Annex IIA 8.5; Annex IIIA 10.7)

B.9.8.4 Summary of studies on non-target micro-organisms – exposure and risk assessment for non-target micro-organisms

Myclobutanil can be evaluated as having no long-term influence on nitrogen transformation (less than 25 % deviation in 28 days), when applied at 2.93 mg Systhane 24 E/kg soil (equivalent to 0.76 mg a.s./kg soil).
Myclobutanil can be evaluated as having no long-term influence on carbon transformation (less than 25 % deviation in 28 days), when applied at 2.93 mg Systhane 24 E/kg soil (equivalent to 0.76 mg a.s./kg soil).
The maximum PEC_{soil} (concentration in soil immediately after last application) is 0.672 mg a.s./kg soil in apples.

In conclusion, the risk of myclobutanil to soil non-target micro-organisms is acceptable.

B.9.10 Effects on biological methods of sewage treatment (Annex IIA 8.7)

Systhane® 20EW: Activated Sludge, Respiration Inhibition Test. (Bürge I., 1999).

Guidelines :
OECD Guideline 209: Activated sludge, respiration inhibition test (1984)
GLP :
Yes
Material and Methods :
Test substance : Systhane 20 EW, formulation containing 20.0 % myclobutanil, batch n°: ES-96018
Test design : The inhibitory effect of myclobutanil on the oxygen consumption of activated sludge suspension (domestic sewage) was determined.
Applied concentrations :
untreated control at start and at end of the test; positive control (3,5-dichlorophenol); treatment at nominal test concentrations of 3.13, 6.25, 12.5, 25, 50, 100 mg a.s./L
1 replicate/treatment, 2 replicates for the control
Test conditions :
microbial inoculum : 3.97 g/L suspended solids
pH : 7.84
temperature : 19.5 – 20.9 °C
Findings :
Inhibitory effects : Inhibition of respiration by the test item was –4, -27, 3, 20, 26 and 70 % for the nominal test concentrations 3.13, 6.25, 12.5, 25, 50, 100 mg a.s./L respectively.
Respiration rates for the two control vessels were comparable (within 6 % of each other).
The calculated EC_{50} of 3,5-dichlorophenol was 10 mg/L, which was within the acceptable limits (5 – 30 mg/L) as specified in the OECD Guideline 209.
Conclusions :
The study is acceptable.
Endpoints :
EC_{50} (myclobutanil, 3 h) = 71 mg a.s./L

ANNEX B

Myclobutanil

B.7 Residue data
(Addendum June 2007)

Data requirement 3.1 : Studies simulating representative processing conditions.

-Processing Study to Determine the Nature of Residues of Myclobutanil Following Industrial or household Preparation ((Rotondaro S.L., 2007)
Guidelines :
EC 7035/VI/95 rev.5
GLP :
Yes
Material and methods :
Test substances : ^{14}C-Myclobutanil and ^{14}C-RH-9090
Specific activity :
- ^{14}C-Myclobutanil : 6.1 mCi/mmol
- ^{14}C-RH-9090 : 3.02 mCi/mmol
Radiochemical purity of the labelled test substance:
- ^{14}C-Myclobutanil : 99.3 %
- ^{14}C-RH-9090 : 93.8 %
Reference standards : Unlabelled myclobutanil, the alcohol metabolite RH-9090, the ketone metabolite RH-9089.
Radioachemical purity for :
-Myclobutanil : 98.75 %
-RH-9090 : 91 %
-RH-9089 : 95.6 %
Preparation of the buffer solutions : A pH 4 buffer was prepared by combining 125 mL citric acid with approximately 100 mL HPLC-grade water. The pH was adjusted to 4.01 with 2 N NaOH. The mixture was transferred to a 250 mL volumetric flask and diluted to volume with HPLC-grade water. The final concentration of the citric acid was 20 mM.
The pH 5 buffer was prepared similarly adjusting the pH to 5.00 with 2 N NaOH prior to diluting to 250 mL.
The pH 6 buffer preparation was similar, adjusting the pH to 6 prior to dilution.
Preparation of the application solution :
A stock solution of each ^{14}C-test substance was prepared by dissolving the test material (0.025 or 0.50 mCi) in 1.0 mL acetonitrile. The stock solutions were stored in a freezer when not in use.
The dosing solutions were prepared by transferring 76 µL of ^{14}C-Myclobutanil stock solution or 19-20 µL of the ^{14}C-RH-9090 stock solution to a 100 mL volumetric flask filled with the appropriate buffer.
The homogeneity and concentration of each dosing solution were determined by LSC. The final concentration of the dosing solutions were 0.85-1.08 µg/mL.
Level of ^{14}C-Myclobutanil in the stock solution : 97.9 %
Level of ^{14}C-RH-9090 in the stock solution : 92.7 %
Experimental design :
The hydrolytic stability of the ^{14}C-Myclobutanil and its metabolite ^{14}C-RH-9090 was investigated in buffered water at pH 4 (20 minutes at 90°C), pH 5 (60 minutes at 100°C) and pH 6 (20 minutes at 120°C) to simulate processing practices.
The samples were analysed directly by LSC and reverse phase HPLC by co-chromatography with reference standards.
3 replicates per set of hydrolysis conditions were prepared for a total of 9 samples per test substance and 18 samples overall.
The treated dosed solution (10 mL) was pipetted into each labelled vial. The pH of each bulk dosing solution was measured and the purity of the bulk solution was verified by HPLC.
No extraction procedure was used.

Findings :

Table 1 : Levels of radioactivity in the buffer solutions during incubation and hydrolysis nature of the residue of ^{14}C-Myclobutanil after heating at different conditions of pH/time/temperature simulating processing practices. The results are expressed in dpm and in percent of the radioactivity initially applied for the 3 replicates.

Simulated process	**Pasteurisation**	**Baking, brewing, boiling**	**Sterilisation**
Buffer solutions	**PH 4 (90°C)**	**PH 5 (100°C)**	**PH 6 (120°C)**
Incubation time (min.)	**20**	**60**	**20**
Radioactivity applied to each replicate (dpm)	432229	463908	449567
Radioactivity recovered in solution after heating (dpm)	425526.93 414711.80 419443.67	456268.73 455824.27 458590.93	424048.67 429761.20 433333.47
Material balance (%)	98.4 95.9 97.0	98.4 98.3 98.9	94.3 95.6 96.4
Average/Standard deviation (%)	*97.1 +/- 1.3*	*98.5 +/- 0.3*	95.4 +/- 1.0
Parent compound analysed by HPLC (%)	97.7 97.7 97.4	97.9 97.6 98.1	97.5 97.4 97.6
Average/Standard deviation (%)	*97.6 +/- 0.2*	*97.9 +/- 0.3*	*97.5 +/- 0.1*
Parent compound analysed by HPLC in the dose solution	96.9	97.6	97.2

Table 2 : Levels of radioactivity in the buffer solutions during incubation and hydrolysis nature of the residue of ^{14}C-RH-9090 after heating at different conditions of pH/time/temperature simulating processing practices. The results are expressed in dpm and in percent of the radioactivity initially applied for the 3 replicates.

Simulated process	**Pasteurisation**	**Baking, brewing, boiling**	**Sterilisation**
Buffer solutions	**PH 4 (90°C)**	**PH 5 (100°C)**	**PH 6 (120°C)**
Incubation time (min.)	**20**	**60**	**20**
Radioactivity applied to each replicate (dpm)	237691	186737	225037
Radioactivity recovered in solution after heating (dpm)	236825.33 239453.07 243209.73	192851.53 192306.47 185713.20	214213.67 211313.33 226308.53
Material balance (%)	99.6 100.7 102.3	103.3 103.0 99.5	95.2 93.9 100.6
Average/Standard deviation (%)	*100.9 +/- 1.3*	*101.9 +/- 2.1*	96.6 +/- 3.5
RH-9090 analysed by HPLC (%)	91.8 92.9 93.2	91.9 92.7 92.8	92.9 93.4 92.4
Average/Standard deviation (%)	*92.6 +/- 0.7*	*92.5 +/- 0.5*	*92.9 +/- 0.5*
Parent compound analysed by HPLC in	92.5	91.9	91.9

the dose solution			

Samples were analysed by LSC and HPLC on the day of the processing and therefore no storage stability data were required.

The pH of each bulk dosing solution and each heated sample was confirmed as unchanged throughout the experiment.
The material balance for Myclobutanil ranged between 95.4 % +/- 1.0% and 98.5 % +/- 0.3 % and for RH-9090 ranged between 96.6 % +/- 3.5% and 101.9 % +/- 2.1%. These results showed that the radioactivity did not dissipate from the test systems during the processing.
In all heated replicates, 100.7, 100.3 and 100.3 % of the radioactivity remained as ^{14}C-Myclobutanil (calculated as the following ratio : average replicates of parent compound by HPLC/dose solution of parent by HPLC).
With regards to the metabolite RH-9090, 100.1, 100.6 and 101.1 % of the radioactivity remained as ^{14}C-RH-9090 in all heated replicates considering the similar ratio as defined for the parent compound.
These values take into account the purity of the dose solution.
Conclusion :
After heating using the different conditions of hydrolysis simulating the processing practices, ^{14}C-Myclobutanil and its metabolite ^{14}C-RH-9090 can be regarded as stable to hydrolysis.

References relied on

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
IIA 6.5	Rotondaro S.L.	2007	Processing Study to Determine the Nature of Residues of Myclobutanil Following Industrial or Household Preparation	N	DAS

Open point 3.20 : Recalculation of livestock dietary burden

Intake calculations for dairy cattle (maximum daily intake of dry matter : 20 kg for 550 kg body weight).

Material	**% of total DM/day**	**Intake of DM from material (kg/animal/day)**	**% dry matter in material**	**Intake of fresh material (kg/animal/day)**	**Residue in material (mg/kg)**	**Residue intake (mg/animal/day)**	**Intake by crop**
Apple pomace (wet)	10	2	23	8.69	1.128	9.80	9.80
Mg/animal/day :							**9.80**
Mg/kg bw/day :							**0.0178**
Mg/kg diet :							**0.494**
Highest residue value of myclobutanil and its alcohol metabolite RH-9090 recovered in the residue trials for apple whole fruit : 0.380 + 0.02 ppm Average Transfer factor for apple wet pomace is 2.97 for myclobutanil.							

Intake calculations for beef cattle (maximum daily intake of dry matter : 15 kg for 350 kg body weight).

Material	**% of total DM/day**	**Intake of DM from material (kg/animal/day)**	**% dry matter in material**	**Intake of fresh material (kg/animal/day)**	**Residue in material (mg/kg)**	**Residue intake (mg/animal/day)**	**Intake by crop**
Apple pomace (wet)	30	4.5	23	19.56	1.128	22.06	22.06
Mg/animal/day :							**22.06**
Mg/kg bw/day :							**0.063**

Mg/kg diet :	**1.5**
Highest residue value of myclobutanil and its alcohol metabolite RH-9090 recovered in the residue trials for apple whole fruit : 0.380 + 0.02 ppm Average Transfer factor for apple wet pomace is 2.97 for myclobutanil.	

Open points 3.17 & 3.18 : Results of trials in apples and grapes additionally accepted as valid by RMS to be presented in an addendum

Supervised residue trials summary sheets :

RESIDUES DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Active substance (common name):	**Myclobutanil**	Commercial Product (name):	**Thiocur 12 EC**
Crop/crop group:	**Apple / Pome Fruit**		
Responsible body for reporting (name & address):	Dow AgroSciences European Development Centre 2nd Floor, 3 Milton Park Abingdon, Oxon. OX14 4RN, UK	Producer of commercial product	**Dow AgroSciences**
Country:	Spain	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/l):	125 g/L	Other active substance in the formulation (common name and content):	None
Formulation (e.g. WP):	EC	Residues calculated as:	Myclobutanil, RH-9090 (mg/kg)
	IIA 6.3.1/07	Masterfile Reference:	ER R86.12

1 Report No. Location (region)	2 Commodity /Variety (a)	3 Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	4 Method of Treatment (c)	5 Application rate per treatment kg a.s./hL	Water (L/ha)	kg a.s./ha	6 Dates of Treatment(s) or No. of treatment(s) and last date (d)	7 Growth stage at last treatment or date (e)	8 Portion analysed (a)	9 Residues (mg/kg) Parent : Myclobutanil	Total RH-9090	10 PHI (days) (f)	11 Remarks: (g)
ER R 86.12 Sudanel, Spain (SZ)	Apples - Golden Delicious	1) 1972 3) 01-09-93	High volume spray – Manual Sprayer	**0.0054** **0.0064** **0.0056** **0.0056**	1398 1166 1340 1340	**0.075** **0.075** **0.075** 0.075	4 treatments: 07-07-93 21-07-93 04-08-93 18-08-93	Fruit Ripening	Whole fruit	0.320 0.129 **0.196**	0.025 0.024 **0.027**	0 14 21	Trial No. 491 93 56 Analytical method: TR 34S-88-10 LOQ (both analytes) = 0.01 mg/kg Sample analysis completed: 31-01-94

(a) According to EEC and Codex classifications (both) should be used.
(b) Only if relevant.
(c) High or low volume spraying, spreading, dusting etc, overall, broadcast, - type of
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4
(f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: climatic conditions: references to analytical method; information concerning the metabolites included,

equipment must be indicated.
(d) Year must be indicated.
Note: All entries to be filled as appropriate

method of storage, storage stability, analysis date and analytical method.

N/A - Not applicable ND - Not detected

RESIDUES DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Active substance (common name):	**Myclobutanil**	Commercial Product (name):	**Systhane 6W**
Crop/crop group:	**Apple / Pome Fruit**		
Responsible body for reporting (name & address):	Dow AgroSciences European Development Centre 2nd Floor, 3 Milton Park Abingdon, Oxon. OX14 4RN, UK	Producer of commercial product	**Dow AgroSciences**
Country:	Germany	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/l):	60 g/kg	Other active substance in the formulation (common name and content):	None
Formulation (e.g. WP):	WP	Residues calculated as:	Myclobutanil, RH-9090 (mg/kg)
	IIA 6.3.1/03	Masterfile Reference:	ER R75.14

1 Report No. Location (region)	2 Commodity /Variety (a)	3 Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	4 Method of Treatment (c)	5 Application rate per treatment			6 Dates of Treatment(s) or No. of treatment(s) and last date (d)	7 Growth stage at last treatment or date (e)	8 Portion analysed (a)	9 Residues (mg/kg)		10 PHI (days) (f)	11 Remarks: (g)
				kg a.s./hL	Water (L/ha)	kg a.s./ha				Parent : Myclobutanil	Total RH-9090		
ER R75.14 2091, Elbstorf, Hamburg, Germany (NZ)	Apples - Golden Delicious	1) 1966 3) 22-09-86	Low volume spray	0.006 0.006 0.018 0.018 0.018 0.018 0.018 0.018 0.018 0.018 0.018 0.018	600 900 400 400 500 500 500 500 500 500 500 500	**0.036** **0.054** **0.072** **0.072** **0.090** **0.090** **0.090** **0.090** **0.090** **0.090** **0.090** **0.090**	21-04-86 29-04-86 09-05-86 19-05-86 29-05-86 12-06-86 24-06-86 09-07-86 25-07-86 10-08-86 24-08-86 08-09-86	Fruit enlargement /ripening	Whole fruit	0.251 0.214 0.089 **0.160** 0.095	<0.01 <0.01 <0.01 **<0.01** <0.01	0 7 14 21 28	Trial No. DEU86F21211 Analytical method: DFG metohd S19 (GC/N-FID); LOQ for : – Parent : 0.005 mg/kg – – RH-9090 : 0.01 mg/kg

(a) According to EEC and Codex classifications (both) should be used.
(b) Only if relevant.
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4
(f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)

(c) High or low volume spraying, spreading, dusting etc, overall, broadcast, - type of equipment must be indicated.
(d) Year must be indicated.
Note: All entries to be filled as appropriate

(g) Remarks may include: climatic conditions: references to analytical method; information concerning the metabolites included, method of storage, storage stability, analysis date and analytical method.

N/A - Not applicable ND - Not detected

Active substance (common name):	**Myclobutanil**	Commercial Product (name):	**Systhane 6W**
Crop/crop group:	**Apple / Pome Fruit**		
Responsible body for reporting (name & address):	Dow AgroSciences European Development Centre 2nd Floor, 3 Milton Park Abingdon, Oxon. OX14 4RN, UK	Producer of commercial product	**Dow AgroSciences**
Country:	Germany	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/l):	60 g/kg	Other active substance in the formulation (common name and content):	None
Formulation (e.g. WP):	WP IIA 6.3.1/03	Residues calculated as: Masterfile Reference:	Myclobutanil, RH-9090 (mg/kg) ER R75.14

1	2	3	4	5			6	7	8	9		10	11
Report No. Location (region)	Commodity /Variety	Date of 1) Sowing or Planting 2) Flowering 3) Harvest	Method of Treatment	Application rate per treatment			Dates of Treatment(s) or No. of treatment(s) and last date	Growth stage at last treatment or date	Portion analysed (a)	Residues (mg/kg)		PHI (days)	Remarks:
	(a)	(b)	(c)	kg a.s./hL	Water (L/ha)	kg a.s./ha	(d)	(e)		Parent : Myclobutanil	Total RH-9090	(f)	(g)
ER R75.14	Apples - Golden	1) 1970	Low	0.006	600	**0.036**	21-04-86	Fruit	Whole fruit	0.205	<0.01	0	Trial No.
	Delicious		volume	0.006	900	**0.054**	30-04-86	ripening		0.183	<0.01	7	DEU86F21221
6520,		3) 13-10-86	spray	0.018	400	**0.072**	09-05-86			0.145	<0.01	14	Analytical method:
Pfeddersheim,				0.018	400	**0.072**	20-05-86			**0.160**	**<0.01**	21	DFG metohd
Germany				0.018	500	**0.090**	02-06-86			0.125	<0.01	28	S19(GC/N-FID);
(NZ)				0.018	500	**0.090**	12-06-86						LOQ for :
				0.018	500	**0.090**	23-06-86		Wet pomace	0.080	<0.01	14	– Parent : 0.005 mg/kg
				0.018	500	**0.090**	09-07-86						
				0.018	500	**0.090**	22-07-86		Juice	0.024	<0.01	14	– RH-9090 : 0.01 mg/kg
				0.018	500	**0.090**	09-08-86						
				0.018	500	**0.090**	01-09-86						
				0.018	500	**0.090**	29-09-86						

(a) According to EEC and Codex classifications (both) should be used.
(b) Only if relevant.
(c) High or low volume spraying, spreading, dusting etc, overall, broadcast, - type of equipment must be indicated.
(d) Year must be indicated.
Note: All entries to be filled as appropriate

(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4
(f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: climatic conditions: references to analytical method; information concerning the metabolites included, method of storage, storage stability, analysis date and analytical method.

N/A - Not applicable ND - Not detected

Active substance (common name):	**Myclobutanil**	Commercial Product (name):	**Systhane 20 EW**
Crop/crop group:	**Apple**		
Responsible body for reporting (name & address):	Dow AgroSciences European Development Centre 2nd Floor, 3 Milton Park Abingdon, Oxon. OX14 4RN, UK	Producer of commercial product	**Dow AgroSciences**
Country:	Northern France	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/l):	200 g/L	Other active substance in the formulation (common name and content):	None
Formulation (e.g. WP):	EW **(GF-1317)** IIA 6.3.1/10	Residues calculated as: Masterfile Reference:	Myclobutanil, RH-9090 (mg/kg) 106.9

1	2	3	4	5			6	7	8	9		10	11
Report No. Location (region)	Commodity /Variety (a)	Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	Method of Treatment (c)	Application rate per treatment			Dates of Treatment(s) or No. of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed (a)	Residues (mg/kg)		PHI (days) (f)	Remarks: (g)
				kg a.s./hL	Water (L/ha)	kg a.s./ha				Parent : Myclobutanil	Total RH-9090		
AF/8164/DE/5 GHE-P-10967 71240 Varennes Le Grand, France (NZ)	Apple – Elstar	1) 1980 3) 20 Sep 04	High volume spray – Airblast Sprayer	0.006 0.006 0.006 0.006 0.006 0.006 0.006 0.006 0.006 0.006 0.006	1417 1381 1478 1552 1374 1449 1548 1568 1476 1437 1575 1535	0.085 0.083 0.089 0.093 0.082 0.087 0.093 0.094 0.089 0.086 0.095 0.092	12 treatments 19 May 04 28 May 04 08 Jun 04 18 Jun 04 28 Jun 04 09 Jul 04 19 Jul 04 27 Jul 04 06 Aug 04 18 Aug 04 26 Aug 04 06 Sep 04	BBCH 79	Fruit	0.15 0.19 0.16 0.16 **0.15** 0.10	0.02 0.01 0.02 0.02 **0.03** 0.02	-0 0 7 14 28 35	Analytical method: myclobutanil/grape/ DMK/03/1 LOQ (both analytes) = 0.01 mg/kg Sample to analysis interval 67 to 102 days

(a) According to EEC and Codex classifications (both) should be used.
(b) Only if relevant.
(c) High or low volume spraying, spreading, dusting etc, overall, broadcast, - type of equipment must be indicated.
(d) Year must be indicated.
Note: All entries to be filled as appropriate

(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4
(f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: climatic conditions: references to analytical method; information concerning the metabolites included, method of storage, storage stability, analysis date and analytical method.

N/A - Not applicable ND - Not detected

Active substance (common name):	**Myclobutanil**	Commercial Product (name):	**Systhane 20EW**
Crop/crop group:	**Grapes**		
Responsible body for reporting (name & address):	Dow AgroSciences European Development Centre 2nd Floor, 3 Milton Park Abingdon, Oxon. OX14 4RN, UK	Producer of commercial product	**Dow AgroSciences**
Country:	Germany	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/l):	200 g/L	Other active substance in the formulation (common name and content):	None
Formulation (e.g. WP):	EW	Residues calculated as:	Myclobutanil, RH-9090 (mg/kg)
	IIA 6.3.2/01	Masterfile Reference:	ER R95.4

1 Report No. Location (region)	2 Commodity/ Variety (a)	3 Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	4 Method of Treatment (c)	5 Application rate per treatment			6 Dates of Treatment(s) or No. of treatment(s) and last date (d)	7 Growth stage at last treatment or date (e)	8 Portion analysed (a)	9 Residues (mg/kg)		10 PHI (days) (f)	11 Remarks: (g)
				kg a.s./hL	Water (L/ha)	kg a.s./ha				Parent : Myclobutanil	Total RH-9090		
ER R95.4 D-67150 Niederkirchen, Germany (NZ)	Grapes - Portugieser	1) 1977 2) 15-06-96 3) 18-09-96	High volume spray to "runoff"- Motorized knapsack sprayer	**0.003** **0.003** **0.003** **0.003** **0.003** **0.003** **0.003** **0.003**	406 783 1004 1139 1326 1461 1573 1600	0.012 0.024 0.030 0.034 0.040 0.044 0.047 0.048	8 treatments: 11-06-96 21-06-96 07-07-96 18-07-96 28-07-96 09-08-96 23-08-96 04-09-96	BBCH 85 Fruit Ripening	Whole Fruit Juice (must) Young wine Mature wine	0.46 0.34 **0.33** 0.07 0.04 0.04	<0.01 0.01 **0.02** <0.01 <0.01 <0.01	0 14 28 14 14 14	Trial No. R&H/203/3/G Analytical method: TR 310-84-13; LOQ (both analytes) = 0.01 mg/kg, Sample to analysis interval ≤ 152 days

(a) According to EEC and Codex classifications (both) should be used.
(b) Only if relevant.
(c) High or low volume spraying, spreading, dusting etc, overall, broadcast, - type of equipment must be indicated.
(d) Year must be indicated.
Note: All entries to be filled as appropriate
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4
(f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: climatic conditions: references to analytical method; information concerning the metabolites included, method of storage, storage stability, analysis date and analytical method.

N/A - Not applicable ND - Not detected (less than 25% of the LOQ)

Active substance (common name):	**Myclobutanil**	Commercial Product (name):	**Systhane 24E**
Crop/crop group:	**Grapes**		

Belgium

Responsible body for reporting (name & address):	Dow AgroSciences European Development Centre 2nd Floor, 3 Milton Park Abingdon, Oxon. OX14 4RN, UK	Producer of commercial product	**Dow AgroSciences**
Country:	France	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/l):	240 g/L	Other active substance in the formulation (common name and content):	None
Formulation (e.g. WP):	EC	Residues calculated as:	Myclobutanil, RH-9090 (mg/kg)
	IIA 6.3.2/03	Masterfile Reference:	ER R96.2

1 Report No. Location (region)	2 Commodity/ Variety (a)	3 Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	4 Method of Treatment (c)	5 Application rate per treatment			6 Dates of Treatment(s) or No. of treatment(s) and last date (d)	7 Growth stage at last treatment or date (e)	8 Portion analysed (a)	9 Residues (mg/kg)		10 PHI (days) (f)	11 Remarks: (g)
				kg a.s./hL	Water (L/ha)	kg a.s./ha				Parent : Myclobutanil	Total RH-9090		
ER R96.2 D-67150 Niederkirchen, Germany (NZ)	Grapes - Muller - Thurgau	2) 16-06-96 3) 16-09-96	Low volume spray – motorized knapsack sprayer	0.009 0.009 0.009 0.009 0.009 0.009 0.009 0.009	334 351 393 379 419 506 504 525	**0.030** **0.032** **0.035** **0.034** **0.038** **0.046** **0.045** **0.047**	8 treatments: 11-06-96 21-06-96 07-07-96 18-07-96 29-07-96 09-08-96 23-08-96 02-09-96	BBCH 83 Fruit Ripening	Whole Fruit	0.48 0.27 **0.29**	0.01 <0.01 **0.01**	0 14 28	Trial No. R&H/202/2/G Analytical method: TR 310-84-13; LOQ (both analytes) = 0.01 mg/kg, Sample to analysis interval ≤ 134 days **Same study as residue trial n° R&H/203/2/G.**

(a) According to EEC and Codex classifications (both) should be used.
(b) Only if relevant.
(c) High or low volume spraying, spreading, dusting etc, overall, broadcast, - type of equipment must be indicated.
(d) Year must be indicated.
Note: All entries to be filled as appropriate

(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4
(f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: climatic conditions: references to analytical method; information concerning the metabolites included, method of storage, storage stability, analysis date and analytical method.

N/A - Not applicable ND - Not detected (less than 25% of the LOQ)

Active substance (common name):	**Myclobutanil**	Commercial Product (name):	**Systhane 24E**
Crop/crop group:	**Grapes**		

Responsible body for reporting (name & address):	Dow AgroSciences European Development Centre 2nd Floor, 3 Milton Park Abingdon, Oxon. OX14 4RN, UK	Producer of commercial product	**Dow AgroSciences**
Country:	France	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/l):	240 g/L	Other active substance in the formulation (common name and content):	None
Formulation (e.g. WP):	EC	Residues calculated as:	Myclobutanil, RH-9090 (mg/kg)
	IIA 6.3.2/03	Masterfile Reference:	ER R96.2

1 Report No. Location (region)	2 Commodity/ Variety (a)	3 Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	4 Method of Treatment (c)	5 Application rate per treatment			6 Dates of Treatment(s) or No. of treatment(s) and last date (d)	7 Growth stage at last treatment or date (e)	8 Portion analysed (a)	9 Residues (mg/kg)		10 PHI (days) (f)	11 Remarks: (g)
				kg a.s./hL	Water (L/ha)	kg a.s./ha				Parent : Myclobutanil	Total RH-9090		
ER R96.2 D-74360 Schozach, Germany (NZ)	Grapes - Spatburgunder	2) 18-06-96 3) 21-09-96	Low volume spray – motorized knapsack sprayer	0.009 0.009 0.009 0.009 0.009 0.009 0.009 0.009	319 338 370 379 417 460 458 533	**0.029** **0.030** **0.033** **0.034** **0.038** **0.041** **0.041** **0.048**	8 treatments: 10-06-96 20-06-96 03-07-96 17-07-96 30-07-96 14-08-96 27-08-96 07-09-96	BBCH 83 Fruit Ripening	Whole Fruit	0.41 0.21 **0.20**	0.01 0.01 **0.02**	0 14 28	Trial No. R&H/202/4/G Analytical method: TR 310-84-13; LOQ (both analytes) = 0.01 mg/kg, Sample to analysis interval ≤ 134 days **Same study as residue trial n° R&H/203/4/G.**

(a) According to EEC and Codex classifications (both) should be used.
(b) Only if relevant.
(c) High or low volume spraying, spreading, dusting etc, overall, broadcast, - type of equipment must be indicated.
(d) Year must be indicated.
Note: All entries to be filled as appropriate
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4
(f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: climatic conditions: references to analytical method; information concerning the metabolites included, method of storage, storage stability, analysis date and analytical method.

N/A - Not applicable ND - Not detected (less than 25% of the LOQ)

Active substance (common name):	**Myclobutanil**	Commercial Product (name):	**Systhane 20 EW**
Crop/crop group:	**Wine Grape / Grapes**		

Responsible body for reporting (name & address):	Dow AgroSciences European Development Centre 2nd Floor, 3 Milton Park Abingdon, Oxon. OX14 4RN, UK	Producer of commercial product	**Dow AgroSciences**
Country:	Northern France	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/l):	200 g/L	Other active substance in the formulation (common name and content):	None
Formulation (e.g. WP):	EW **(GF-1317)**	Residues calculated as:	Myclobutanil, RH-9090 (mg/kg)
	IIA 6.3.2/08	Masterfile Reference:	106.11

1	2	3	4	5			6	7	8	9		10	11
Report No. Location (region)	Commodity /Variety (a)	Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	Method of Treatment (c)	Application rate per treatment			Dates of Treatment(s) or No. of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed (a)	Residues (mg/kg)		PHI (days) (f)	Remarks: (g)
				kg a.s./hL	Water (L/ha)	kg a.s./ha				Parent : Myclobutanil	Total RH-9090		
AF/8165/DE/1 GHE-P-10966 71700 Uchizy, France (NZ)	Wine grape - Chardonnay	1) 1987 3) 06 Sep 04	High volume spray – Airblast Sprayer	0.0048 0.0048 0.0048 0.0048	1083 1011 1040 997	0.052 0.049 0.050 0.048	4 treatments 23 Jul 04 03 Aug 04 13 Aug 04 23 Aug 04	BBCH 81	Grape bunches	0.06 0.07 0.06 0.04 **0.05** 0.02	<0.01 <0.01 <0.01 <0.01 **<0.01** <0.01	-0 0 7 14 28 35	Analytical method: myclobutanil/grape/ DMK/03/1 LOQ (both analytes) = 0.01 mg/kg Sample to analysis interval 31-66 days

(a) According to EEC and Codex classifications (both) should be used.
(b) Only if relevant.
(c) High or low volume spraying, spreading, dusting etc, overall, broadcast, - type of equipment must be indicated.
(d) Year must be indicated.
Note: All entries to be filled as appropriate

(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4
(f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: climatic conditions: references to analytical method; information concerning the metabolites included, method of storage, storage stability, analysis date and analytical method.

N/A - Not applicable ND - Not detected

Active substance (common name):	**Myclobutanil**	Commercial Product (name):	**Systhane 20 EW**
Crop/crop group:	**Wine Grape / Grapes**		

Responsible body for reporting (name & address):	Dow AgroSciences European Development Centre 2nd Floor, 3 Milton Park Abingdon, Oxon. OX14 4RN, UK	Producer of commercial product	**Dow AgroSciences**
Country:	Northern France	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/l):	200 g/L	Other active substance in the formulation (common name and content):	None
Formulation (e.g. WP):	EW **(GF-1317)** IIA 6.3.2/08	Residues calculated as: Masterfile Reference:	Myclobutanil, RH-9090 (mg/kg) 106.11

1	2	3	4	5			6	7	8	9		10	11
Report No. Location (region)	Commodity /Variety (a)	Date of 1) Sowing or Planting 2) Flowering 3) Harvest (b)	Method of Treatment (c)	Application rate per treatment			Dates of Treatment(s) or No. of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed (a)	Residues (mg/kg)		PHI (days) (f)	Remarks: (g)
				kg a.s./hL	Water (L/ha)	kg a.s./ha				Parent : Myclobutanil	Total RH-9090		
AF/8165/DE/2 GHE-P-10966 71260 St Pierre de Lanques, France (NZ)	Wine grape - Chardonnay	1) 10 May 83 3) 06 Sep 04	High volume spray – Airblast Sprayer	0.0048 0.0048 0.0048 0.0048 0.0048 0.0048 0.0048 0.0048	1088 1006 990 925 1039 974 1023 974	0.052 0.048 0.048 0.044 0.050 0.047 0.049 0.047	8 treatments 14 Jun 04 22 Jun 04 02 Jul 04 13 Jul 04 23 Jul 04 03 Aug 04 13 Aug 04 23 Aug 04	BBCH 81	Grape bunches	0.18 0.41 0.07 0.08 **0.10** 0.11	0.03 0.03 <0.01 0.01 **0.02** 0.01	-0 0 7 14 28 35	Analytical method: myclobutanil/grape/ DMK/03/1 LOQ (both analytes) = 0.01 mg/kg Sample to analysis interval 37-72 days

(a) According to EEC and Codex classifications (both) should be used.
(b) Only if relevant.
(c) High or low volume spraying, spreading, dusting etc, overall, broadcast, - type of equipment must be indicated.
(d) Year must be indicated.
Note: All entries to be filled as appropriate

(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4
(f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: climatic conditions: references to analytical method; information concerning the metabolites included, method of storage, storage stability, analysis date and analytical method.

N/A - Not applicable ND - Not detected

Active substance (common name):	**Myclobutanil**	Commercial Product (name):	**Systhane 20 EW**
Crop/crop group:	**Table grape / Grapes**		

Responsible body for reporting (name & address):	Dow AgroSciences European Development Centre 2nd Floor, 3 Milton Park Abingdon, Oxon. OX14 4RN, UK	Producer of commercial product	**Dow AgroSciences**
Country:	Spain	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/l):	200 g/L	Other active substance in the formulation (common name and content):	None
Formulation (e.g. WP):	EW **(GF-1317)**	Residues calculated as:	Myclobutanil, RH-9090 (mg/kg)
	IIA 6.3.2/09	Masterfile Reference:	106.12

1	2	3	4	5			6	7	8	9		10	11
Report No. Location (region)	Commodity /Variety	Date of 1) Sowing or Planting 2) Flowering 3) Harvest	Method of Treatment	Application rate per treatment			Dates of Treatment(s) or No. of treatment(s) and last date	Growth stage at last treatment or date	Portion analysed (a)	Residues (mg/kg)		PHI (days)	Remarks:
	(a)	(b)	(c)	kg a.s./hL	Water (L/ha)	kg a.s./ha	(d)	(e)		Parent : Myclobutanil	Total RH-9090	(f)	(g)
AF/7779/DE/3 GHE-P-10965 41820 Seville, Spain (SZ)	Table grapes - Regina	1) 03-1997 3) 10 Aug 04	High volume spray – to 1000l/ha - Airblast Sprayer	0.0048 0.0048 0.0048 0.0048 0.0048 0.0048	917 1038 968 967 951 1000	0.044 0.050 0.046 0.046 0.046 0.048	6 treatments 07 Jun 04 17 Jun 04 28 Jun 04 07 Jul 04 16 Jul 04 27 Jul 04	BBCH 81-83	Grape bunches	<0.01 0.35 0.12 0.10 **0.08** 0.04	<0.01 0.02 0.02 0.02 **0.03** 0.02	-0 0 7 14 28 35	Analytical method: myclobutanil/grape/ DMK/03/1 LOQ (both analytes) = 0.01 mg/kg Sample to analysis interval 69-134 days

(a) According to EEC and Codex classifications (both) should be used.
(b) Only if relevant.
(c) High or low volume spraying, spreading, dusting etc, overall, broadcast, - type of equipment must be indicated.
(d) Year must be indicated.
Note: All entries to be filled as appropriate

(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4
(f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: climatic conditions: references to analytical method; information concerning the metabolites included, method of storage, storage stability, analysis date and analytical method.

N/A - Not applicable ND - Not detected

Myclobutanil - B.6 Toxicology and metabolism

EFSA ADDENDUM (16 November 2007)

REASON FOR THE ADDENDUM

During PRAPeR 19, the RMS (BE) was asked to re-calculate operator, worker and bystander exposure based on the agreed dermal absorption values.

An amended addendum ("Post PRAPeR 19) has been submitted in March 2007.

During the commenting phase on the EFSA draft conclusion and during the Evaluation Meeting held in Parma on 14-15 November 2007, some inaccuracies have been highlighted for operator and worker exposure estimates.

Therefore, after the meeting it was decided to revise calculations in order to provide the correct assessment. It is noted that re-calculations presented in the EFSA addendum did not change the final conclusion on the risk assessment, with regard to the safety of intended uses.

The lay out and general information on the exposure for operators and workers have been taken from the last addendum provided by Belgium (March 2007, Post PRAPeR 19).

Systhane 20 EW is an emulsion (oil in water) formulation, containing a nominal 200-g/L myclobutanil. It is applied at a maximum individual rate of 90 g a.s./ha during the fruit/grain growth/ripening and the maximum duration of the application season will be less than three months. Water is the intended diluent/carrier.

Dermal absorption: 25% of the concentrate and 15% of the diluted formulation respectively.

Predicted exposure is compared with the systemic AOEL = 0.03 mg/kg bw/d.

UK POEM scenario:

tractor mounted broadcast air-assisted sprayer (500 l/ha)

treated area: 15 ha

German model:

tractor high crops application scenarios

treated area 8 ha

Applications parameters as proposed by the company:

Table B.6.15.1-1: Application information on representative crops.

Crop	**Application method**	**Max. dose rate L product/ha**	**Max.dose rate G active substance/ha**	**Spray volume L/ha**	**Pack size L**
Grape	Air-assisted low and high water volume	0.048	48	1000	1
Apple	Air-assisted low and high water volume	0.09	90	1000	1

Predicted operator exposures:

UK POEM: tractor mounted broadcast air assisted sprayer 500 L/ha model- apples, no PPE

Product data

Product	Systhane 20 EW
Active substance	myclobutanil
Concentration	200 mg/ml
Formulation type	EC
Maximum in use a.s.concentration	0.018 mg/ml

Exposure during mixing and loading

Container size	1 L
Hand contamination/operation	0.01 ml
Application dose	0.09 L product/ha
Work rate	15 ha/day
Number of operations	2 day
Hand contamination	0.02 g/day
Protective clothing	None
Transmission to skin	100%
Dermal exposure to formulation	0. 02 ml/day

Exposure during spray application

Application volume	1000 L spray/ha		
Volume of surface contamination	400 ml/h		
Distribution	Hands	Trunk	Leggs
	10	65	25%
Clothing	None	Permeable	Permeable
	100	2	5%
Dermal exposure	10	5.2	5 ml/h
Duration of exposure	6 h		
Total dermal exposure to spray	121.2 ml/day		

Absorbed dose	Mix/load	Application
Dermal exposure	0.02 ml/day	121.25 ml/day
Concentration of a.s.	200 mg/ml	0.018 mg/ml
Dermal exposure to a.s.	4 mg/day	2.1816 mg/day
Percent absorbed	25%	15%
Absorbed dose	1 mg/day	0.32724 mg/day

Inhalation exposure during spraying

Inhalation exposure	0.05 ml/h
Duration of exposure	6 h
Concentration of a.s.	0.018 mg/ml
Inhalation exposure to a.s.	0.0054 mg/day
Percent absorbed	100%
Absorbed dose	0.0054 mg/day

Predicted exposure

Total absorbed dose	1.33264 mg/day
Operator body weight	60 kg

Operator exposure	0.02221 mg/kg bw/day

UK POEM: tractor mounted broadcast air assisted sprayer 500 L/ha model- grapes, no PPE

Product data

Product	Systhane 20 EW
Active substance	myclobutanil
Concentration	200 mg/ml
Formulation type	EC
Maximum in use a.s.concentration	0.0096 mg/ml

Exposure during mixing and loading

Container size	1 L
Hand contamination/operation	0.01 ml
Application dose	0.048 L product/ha
Work rate	15 ha/day
Number of operations	1 day
Hand contamination	0.01 g/day
Protective clothing	None
Transmission to skin	100%
Dermal exposure to formulation	0. 01 ml/day

Exposure during spray application

Application volume	1000 L spray/ha		
Volume of surface contamination	400 ml/h		
Distribution	Hands	Trunk	Leggs
	10	65	25%
Clothing	None	Permeable	Permeable
	100	2	5%
Dermal exposure	10	5.2	5 ml/h
Duration of exposure	6 h		
Total dermal exposure to spray	121.2 ml/day		

Absorbed dose	Mix/load	Application
Dermal exposure	0.01 ml/day	121.25 ml/day
Concentration of a.s.	200 mg/ml	0.0096 mg/ml
Dermal exposure to a.s.	2 mg/day	1.16352 mg/day
Percent absorbed	25%	15%
Absorbed dose	0.51 mg/day	0.174528 mg/day

Inhalation exposure during spraying

Inhalation exposure	0.05 ml/h
Duration of exposure	6 h
Concentration of a.s.	0.0096 mg/ml
Inhalation exposure to a.s.	0.00288 mg/day
Percent absorbed	100%
Absorbed dose	0.00288 mg/day

Predicted exposure

Total absorbed dose	0.677408 mg/day
Operator body weight	60 kg
Operator exposure	0.011290133 mg/kg bw/day

German model: apple

Use information

Product	Systhane 20EW	Active substance	myclobutanil	
Formulation type	liquid	a.s. concentration	200 mg/ml	
Method of use	Tractor high crops	Dose(product)	1000 L product/ha	
Work rate	8 ha/day	Dose (a.s.)	0.09 kg a.s./ha	
		Amount handled	0.72 kg a.s./day	

Exposures-mix/loading

	Specific exposures	Estimated exposures	PPE	Estimated exposures
Inhalation	0.0006 mg/kg a.s.handled	0.000432mg a.s./day	None	0.000432 mg a.s./day
Dermal-hands	2.4 mg/kg a.s.handled	1.728 mg a.s./day	Gloves	0.01728 mg a.s./day

Exposures-application

	Specific exposures	Estimated exposures	PPE	Estimated exposures (PPE)
Inhalation	0.018 mg/kg a.s.handled	0.01296 mg a.s./day	None	0.01296 mg a.s./day
Dermal-head	1.2 mg/kg a.s.handled	0.864 mg a.s./day	None	0.864 mg a.s./day
Dermal –hands	0.7 mg/kg a.s.handled	0.504 mg a.s./day	Gloves	0.00504 mg a.s./day
Dermal- body	9.6 mg/kg a.s.handled	6.912 mg a.s./day	none	6.912 mg a.s./day
Total exposures		Estimated exposures	Percent absorbed	Estimated exposures (PPE)
Total potential inhalation		0.01339 mg a.s./day	100%	0.01339 mg a.s./day
Total dermal-mix		1.728 mg a.s./day	25%	0.01728 mg a.s./day
Total dermal-application		8.28 mg a.s./day	15%	7.781 mg a.s./day
Total absorbed dose		1.6873mg a.s./day		1.1848 mg a.s./day
Body weight		70 kg		70 kg
Mg/kg bw/d		0.0241 mg/kg bw/d		0.01692 mg/kg bw/d

Comparison of estimated and tolerable exposure:

Table B.6.15.1-3: Exposure as a proportion of AOEL 0.03 mg/kg bw/day

Crop/application method	% of AOEL
	No PPE worn
UK POEM model	
Grapes, orchard	37.6%
Apples, orchard	74%
German model	
Grapes, orchard	42%
Apples, orchard	80%

Conclusions: predicted exposure to myclobutanil formulated as Systhane 20 EW was compared with the systemic AOEL = 0.03mg/kg bw/d.

The estimated exposure levels for the operator are below the AOEL for both the German and UK POEM models, even withouth the use of PPE.

B.6.15.2 Measurement of operator exposure (Annex IIIA 7.2.1.2)

No data, not required.

B.6.15.3 Estimation of bystander exposure (Annex IIIA 7.2.2)

See RMS Addendum March 2007 (Post PRAPeR 19)

B.6.15.4 Estimation of worker exposure (Annex IIIA 7.2.3.1)

This assessment considers the potential for exposure resulting from the maximum use rate and immediate re-entry, and assumes that PPE is not used.

It covers both workers and non-worker re-entry.

In all re-entry situations, the low volatility of the active substance (1.98×10^{-4} Pa, at 20°C) removes a concern of exposure to vapour. The major route of exposure on re-entry is contact with residues via the skin. The use of the product that represents the greatest concern is on apple and grapes.

Exposure from contact with a treated crop.

Exposure through re-entry into the crop was calculated below for grapes and apples:

Parameters	Value	Reference
Application rate (g/ha)	90	Label
Deposition rate (ng/cm^2 for g a.s./ha)	3	Poppendorf, 1992
Percent dislogeable	80%	Gunther et al., 1973
Max. Dislogeable foliar residue (mg a.s./cm^2)	0.000216	
Body weight	70 kg	
Transfer factor with gloves (cm^2/h)	5000	US EPA RED Diazinon, 2000
Task duration (hour)	8	Assumed
Percent dermal absorption	15%	See dermal absorption studies
Absorbed dose (mg/kg bw/d)	0.0185	Calculated (see below)
AOEL (mg/kg bw/d)	0.03	See proposal for AOEL
Dose as % of AOEL	61.7%	

Where:

Max.dislogeable folair residue= (application rate) x (deposition rate/1000000) x(percent dislogeable/100)

$$\text{Percent dermal exposure} = \frac{\text{DFR (mg a.s./cm}^2\text{) x transfer coefficient (cm}^2\text{/hr) x task duration (hr/day)}}{\text{Body weight (kg)}}$$

In conclusion, the estimated worker exposure for the highest application rate (apples), shows exposure levels below the AOEL (61.7%).

It is noted the only the exposure occurring after 1 single application has been estimated.

B.6.15.5 Measurement of worker exposure (Annex IIIA 7.2.3.2)

Not necessary, not required.