95% of saturation) over the test duration.

Observations: Mortality and abnormal behaviour was monitored at 24, 48, 72 and 96 hours. Temperature, pH and dissolved oxygen concentrations were recorded daily and water samples, collected at test initiation and test termination, were analysed for test substance concentration by HPLC.

Data analysis: Moving average method, probit method, non-linear interpolation.

Results:

Mean measured difenoconazole concentrations corresponded to 74 – 100% of nominal concentrations. Mortality data are presented in Table B.9.2.1-5.

Table B.9.2.1-5: Effect of difenoconazole on mortality in sheepshead minnow

Mean measured concentration (mg/L)	Cumulative mortality (%)			
	24 hour	48 hour	72 hour	96 hour
Control	0	0	0	0
Solvent control	0	0	0	0
0.27	0	0	0	0
0.4	0	0	0[b]	0[b]
0.86	0	0[b]	0[b]	0a
1.5	0[a]	0[a]	85[a]	100
2.0	100	100	100	100
LC_{50} (mg/L)	1.7	1.7	1.2	1.1
95% confidence interval (p=0.05)	1.5-2.0	1.5-2.0	0.86-1.5	0.86-1.5

[a] *all surviving fish suffering loss of equilibrium*
[b] *one or more fish suffering loss of equilibrium*

Based on mean measured concentrations, the 96-hour LC_{50} for difenoconazole in sheepshead minnow was estimated to be 1.1 mg/L.

RMS comments:

The study was conducted in accordance with the referred guidelines. At the two lowest test levels, the measured concentrations were significantly higher than the initial values, due to a malfunction of the diluter system. Therefore, only the initial measured values were used for these levels when the LC_{50} was calculated. This would not have influenced the results to a significant extent, and the study is considered valid for the risk assessment.

According to OECD Guidelines 203 if the data obtained are not suitable for standard methods of calculation of the LC_{50} (for example cases like this, where the dose response curve goes from 0% to 100% mortality between two subsequent concentrations) then the geometric mean of the highest concentration causing no immobility and the lowest causing 100% immobility can be used as an approximate LC_{50} when the treatment levels do not differ by more than a factor of 2. In this case, this value was identical as the reported value.

METABOLITES

Reference: **Rufli H (1983). Report on the test for acute toxicity of CGA 98032 to rainbow trout. [redacted] Unpublished report no. 821418. (Syngenta File No 71019/0024)**

Guideline:	OECD 203 (1981).
GLP:	Yes.

Material and methods:

Test substance:	Technical CGA71019 (synonymous with CGA 98032, 1,2,4-triazole) 91.9% content, Batch No. EN38530.
Species:	Juvenile rainbow trout, *Onchorynchus mykiss* (mean body length 53 mm; mean body weight 1.27 g)
Treatments:	Nominal test concentrations were 100, 180, 320, 580 and 1000 mg CGA 71019/L Dechlorinated tap water was used to prepare the test solutions.
Number of animals:	The test incorporated two replicate tanks of five fish for each exposure concentration and for the untreated water control.
Duration:	96 hours, static system.
Test conditions:	Temperature 15°C, pH 7.6 – 8.1. 16 hours of light per day. Over the test period dissolved oxygen concentrations ranged between 8.2-10.1 mg/L (>60% of saturation).
Observations:	Mortalities and symptoms of toxicity were recorded at intervals of 24 hours throughout the test up to 96 hours. Dissolved oxygen, temperature, pH were recorded during the study and the concentrations of CGA 71019 in the test systems were determined by gas chromatography (GC) at 0 and 96 hours.
Data analysis:	Graphical determination and calculation according to Finney (1964).

Results:

The test concentrations of CGA 71019, ranged from 55 to 86% of nominal at study start and from 52 to 73% of nominal in the samples taken after 96 hours. Over the exposure period, abnormal swimming behaviour and loss of equilibrium was observed in fish exposed to 180 mg CGA 71019/L and higher, and there were slight effects on pigmentation in the 580 mg/L treatment group.

The LC_{50} values that were presented in this study report were based on nominal concentrations (Table 8.2.1-6). However, as some measured concentrations were <80% of nominal, LC_{50} values were re-calculated based on mean measured values (**Powley, 2003**). These values are also presented in the table below.

Table B.9.2.1-6: Effect of CGA 71019 on mortality in rainbow trout

Concentration [mg/L]		**Mortality [%]**			
Nominal	**Mean Measured**	**24h**	**48h**	**72h**	**96h**
Water	-	0	0	0	0
100	52	0	0	0	0
180	132	0	0	0	0
320	192	0	0	0	0
580	378	0	0	0	0
1000	657	0	90	100	100
LC_{50} (mg/L) based on nominal concentrations		>1000	800	760	760
LC_{50} (mg/L) based on mean measured concentrations (95% C.I. p≤0.05)		>657	528 (n.d.)	498 (378-657)	498 (378-657)

n.d. not determined

Based on mean measured concentrations, the 96-h LC_{50} for CGA 71019 in rainbow trout was 498 mg/L.

RMS comments:

According to OECD Guidelines 203 if the data obtained are not suitable for standard methods of calculation of the LC_{50} (for example cases like this, where the dose response curve goes from 0% to 100% mortality between two subsequent concentrations, and when these concentrations do not differ by more than a factor of 2) then the geometric mean of the highest concentration causing no immobility and the lowest causing 100% immobility can be used as an approximate LC_{50}. In this case, the LC_{50} based on this approach was identical to the value based on mean measured concentrations presented in the table above.

Reference:	**Swarbrick, R.H. (2001a). CGA 205375 – Acute toxicity to rainbow trout (*Oncorhynchus mykiss*). [redacted] Unpublished report no. BL7201/B (Syngenta File No 205375/0014)**
Guideline:	OECD 203.
GLP:	Yes.
Material and methods:	
Test substance:	Technical CGA 205375 (triazolylalcohol), batch number MLA-421/2, purity 99%.
Species:	Rainbow trout (*Oncorhynchus mykiss*)
Treatments:	Technical CGA 205375 was introduced into tanks to produce 5 nominal exposure concentrations (0.12, 0.25, 0.5, 1.0, 2.0 mg/L). The test incorporated one tank for each concentration level and the untreated control tank.
Number of animals:	Ten fish (mean weight 1.98 g; mean length 51 mm) were introduced to each tank.
Duration:	96 hours, static test.
Test conditions:	Temperature 15±1°C, pH 7.4-7.7. 16 hours light per day. The hardness and conductivity of dilution water were 46.7 $CaCO_3$ mg/L and 233 µS/cm, respectively. Dissolved oxygen concentrations varied between and 9.5-10.1 mg/L (95 – 100% of saturation) over the test duration.
Observations:	Mortality and symptoms of toxicity were monitored at 24, 48, 72 and 96 hours. Temperature, pH and dissolved oxygen concentrations were also recorded daily and water samples, collected at test initiation and test termination, were analysed for test substance concentration by HPLC.
Data analysis:	Binominal and moving average method.

Results:

Mean measured difenoconazole concentrations in centrifugated samples corresponded to 44-61% of nominal concentrations. Mortality data are presented in the table below.

Table B.9.2.1-7: Effect of CGA 205375 on mortality in rainbow trout

Mean measured concentration (mg/L)	Cumulative mortality (%)			
	24 hour	48 hour	72 hour	96 hour
Control	0	0	0	0
0.073	0	0	0	0[a]
0.11	0	0	0	0[a]
0.24	0[a]	0[a]	0[a]	0[a]
0.58	0[b]	0[b]	0[b]	0[b]
0.95	10[b]	10[b]	100	100
LC_{50} (mg/L)	>0.95	>0.95	0.74	0.66
95% confidence interval (p=0.05)	n.c.	n.c.	0.58-0.95	0.54-0.85

[a] *10-30% of fish dead or showing symptoms of toxicity*
[b] *>30% of fish dead or showing symptoms of toxicity*
n.c. not calculated

Based on mean measured concentrations, the 96-hour LC_{50} for CGA 205375 in rainbow trout was estimated to be 0.66 mg/L.

RMS comments:

The study was conducted in accordance with the referred guidelines, although more than one replicates would have been preferred. According to OECD Guidelines 203 if the data obtained are not suitable for standard methods of calculation of the LC_{50} (for example cases like this, where the dose response curve goes from 0% to 100% mortality between two subsequent concentrations that do not differ by more than a factor of 2) then the geometric mean of the highest concentration causing no immobility and the lowest causing 100% immobility can be used as an approximate LC_{50}. The calculated LC_{50} based on this approach is 0.74 mg/L. This value will be used in the risk assessment.

FORMULATED PRODUCTS

DIVIDEND 030FS

Reference:	**Gries, T. (1999a). Acute toxicity of CGA 169374 FS (30) (A-9142 G) to rainbow trout (*Oncorhynchus mykiss*). [redacted] Switzerland. Unpublished report no 1047.062.103. Study dates 14-18 June 1999 (Syngenta File No. CGA 169374/1907)**
Guideline:	OECD 203 (1992).
GLP:	Yes.

Material and methods:

Test substance:	A-9142 G containing 30.6 g/L CGA 169374 (density 1049 kg/m^3). Batch number P.902001.
Species:	Rainbow trout (*Oncorhynchus mykiss*).
Treatments:	Aliquots of test substance, prepared in water, were introduced into tanks to produce nominal exposure concentrations of 6.25, 12.5, 25, 50 and 100 mg formulation/L. The test incorporated one tank for each exposure concentration and a control tank of dechlorinated tap water.
Number of animals:	Seven fish (mean weight 0.64 g; mean length 41 mm) were introduced into 14 L of water in each tank.
Duration:	96 hours, static test.

Test conditions:	Temperature 13.5± 1°C, pH 6.6 – 7.5. 16 hours light per day. The hardness and conductivity of dilution water were 180 $CaCO_3$ mg/L and 445 µS/cm, respectively. Dissolved oxygen concentrations ranged between 9.5-11.3 mg/L (94 – 112% of saturation) over the test duration.
Observations:	Fish were monitored at 0, 2, 4, 24, 48, 72 and 96 hours for mortality and behavioural abnormalities. Temperature, pH and dissolved oxygen concentrations were recorded at 24-hour intervals. Test substance concentrations were determined in water samples collected at test initiation and test termination.
Data analysis:	Probit analysis.

Results:

Mean initial test substance concentrations corresponded to 81-96.7% of nominal concentrations. After 96 hours, the concentrations were 36.3 – 68.9% of the nominal values. Mortality data are presented in the table below.

Table B.9.2.1-8: Acute toxicity of A-9142 G to rainbow trout

Nominal concentration (mg/L)	Cumulative mortality (%)			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
6.25	0	0	0	0
12.5	0	0	0	0
25	0*	0*	0*	0*
50	100	100	100	100
100	100	100	100	100
LC_{50} (mg/L)	37.5	37.5	37.5	37.5
95% confidence interval (p=0.05)	n.d.	n.d.	n.d.	n.d.

n.d. not determined as only two data points available for regression analysis
**fish suffering from lethargy, discolouration, abnormal respiration and loss of equilibrium.*

Based on nominal concentrations, the 96-hour LC_{50} value for A-9142 G in rainbow trout was 37.5 mg formulation/L (1.09 mg as/L).

RMS comments:

Only one replicate was used, however this is acceptable according to the referred guidelines. Since the test concentrations declined to less than 80% of the nominal values during the study, the results should be based on mean measured concentrations. Further, according to OECD Guidelines 203 if the data obtained are not suitable for standard methods of calculation of the LC_{50} (for example cases like this, where the dose response curve goes from 0% to 100% mortality between two subsequent concentrations that do not differ by more than a factor of 2) then the geometric mean of the highest concentration causing no immobility and the lowest causing 100% immobility can be used as an approximate LC_{50}. At 25 mg formulation/L, measured concentration of active ingredient was initially 0.591mg as/L and at the end 0.265 mg as/L, with a mean measured of 0.428 mg as/L. At 50 mg/L, the corresponding values were 1.32 mg as/L at start, 1.00 mg as/L at the end, and mean measured was 1.16 mg as/L. The geometric mean of mean measured concentrations was calculated to be 0.70 mg as/L. This value will be used in the risk assessment.

The notifier disagreed with the recalculation of the study endpoints based on mean measured values since it is not in line with the test objective to determine the toxicity of the formulated product. Formulation studies differ from studies on the active substance in that the objective is to determine the toxicity of the whole product (including formulation adjuvants and blanks). In such cases, analysis of the active ingredient serves to demonstrate correct dosing of the product. At the start of the test, measured concentrations were between 80 to 120% of nominal (based on analysis of difenoconazole), thus indicating correct dosing of the product. The notifier stated that in this case it is more appropriate to calculate the results based on nominal concentrations. However, the formulation studies in this case is used to assess the risk from the active ingredient, and for precautionary reasons it is assumed that the total toxicity derives from difenoconazole, the RMS maintains that the results should be based on mean measured concentrations. This is also in line with current practice.

SCORE 250EC

Reference:	**Voigt, H. (1990a). Toxizität von CGD 96430F für Regenbogenforellen (*Salmo gairdnerii*) (48 h) [redacted] Unpublished Report No. 05/90/232, Experimental phase 14 – 18 May 1990. Syngenta File No. CGA169374/0761**
Guideline:	OECD 203 (1984).
GLP:	No, but test was claimed to be conducted to sound scientific principles
Material and methods:	
Test substance:	CGD 96430F(equivalent to A-7402 A), containing 250g/L difenoconazole, batch no. P 901011.
Species:	Rainbow trout (*Oncorhynchus mykiss*)
Treatments:	Juvenile rainbow trout (*Salmo gairdnerii,* syn. *Oncorhynchus mykiss*) were exposed to nominal formulation concentrations of 0.28, 0.56, 1.13, 2.25 and 4.50 mg/L of A-7402 A in a static test design for 96 hours.
Number of animals:	The test incorporated one tank containing 10 fish for the control and for each test concentration.
Duration:	96 hours, static test.
Test conditions:	Water temperature was maintained at 16.6 ± 1°C, pH 7.9 – 8.2. The total hardness of dilution water was 200-240 mg CaCO3/L. Dissolved oxygen concentrations ranged between 85-100% over the test duration.
Observations:	Fish were monitored at 0, 3, 6, 24, 48, 72 and 96 hours for mortality and behavioural abnormalities. Temperature, pH and dissolved oxygen concentrations were recorded in control and 2.25 mg/L tanks at 24-hour intervals and in control and 4.5 mg/L tanks at test initiation. Water samples collected at 2, 48 and 96 hours after test initiation were analysed for test substance concentration in control, 0.28, 0.56 and 2.25 mg/L exposure tanks.
Data analysis:	Not stated.

Results:

Mean measured difenoconazole concentrations corresponded to 104-118% of nominal values 2 hours after test initiation and fell to 62-100% after 96 hours. Mean measured during the study was 82 – 100%, and therefore the results were based on nominal concentrations. Mortality data are presented in the table below.

With the exception of a single dead fish at 0.56 mg/L at 96 hours, there were no mortalities in the control or test treament tanks up to and including 2.25 mg A-7402 A/L. In the test treament tank at 4.50 mg A-7402 A/L, all fish died between 6 and 24 hours after the start of the experiment. Symptoms of toxicity observed in the fish were miscoordination, discoloration and increased respiratory frequency.

Table B.9.1.2-9: Measured concentrations of difenoconazole in the test solutions and fish mortality

Nominal concentration (mg/L)		Difenoconazole (mg/L)			Mortality (%; 96 hours)
CGD 96430F	**difenoconazole**	**2 hours**	**48 hours**	**96 hours**	
Control	0	<0.03	<0.03	<0.03	0
0.28	0.07	0.08	0.07	0.07	0
0.56	0.14	0.17	0.14	0.13	10
1.13	0.28	n.d.	n.d.	n.d.	0
2.25	0.56	0.58	0.42	0.35	0
4.50	1.12	n.d.	n.d.	n.d.	100

n.d. not determined

The 96-hour LC_{50} of CGD 96430F to *Oncorhynchus mykiss* was 3.2 mg/L formulation, equivalent to 0.8 mg as/L based on nominal test concentrations. The value was calculated as the geometric mean of the highest concentration causing no mortality (excluding the single dead fish at 0.56 mg/L, considered not treatment related) and the lowest causing 100% mortality.

RMS comments:

The study report was written in German. At the highest test level, no measurements were reported. Assuming a similar recovery as in the second highest test level, 82%, the LC_{50} based on estimated mean measured concentrations can be calculated to 0.65 mg as/L, or 2.6 mg formulation/L. These values will be used in the risk assessment.

The notifier disagreed with the recalculation of the study endpoints based on mean measured values since is not in line with the test objective to determine the toxicity of the formulated product. Formulation studies differ from studies on the active substance in that the objective is to determine the toxicity of the whole product (including formulation adjuvants and blanks). In such cases, analysis of the active ingredient serves to demonstrate correct dosing of the product. At the start of the test, measured concentrations were between 80 to 120% of nominal (based on analysis of difenoconazole), thus indicating correct dosing of the product. The notifier stated that in this case it is more appropriate to calculate the results based on nominal concentrations. However, the formulation studies in this case is used to assess the risk from the active ingredient, and for precautionary reasons it is assumed that the total toxicity derives from difenoconazole, the RMS maintains that the results should be based on mean measured concentrations. This is also in line with current practice.

Reference:	**Rufli, H. (1994). Report on the acute toxicity test of CGA 169374 (A-7402 H) to Rainbow trout (*Oncorhynchus mykiss*). [redacted] Unpublished report no. 943539. Experimental phase 6 April – 10 May 1994 Syngenta File N° CGA169374/0932**
Guideline:	OECD 203 (1984).
GLP:	Yes

Material and methods:

Test substance:	A-7402 H containing 250g/L difenoconazole; Batch number P.308002; Density 1.0129 kg/L.
Species:	Rainbow trout (*Oncorhynchus mykiss)*
Treatments:	Aliquots of A-7402 H, prepared in water, were introduced into tanks to produce nominal exposure concentrations of 0.5, 1.1, 2.3, 5 and 11 mg formulation/L. The test incorporated one tank for each exposure concentration and a control tank of dechlorinated tap water.
Number of animals:	Seven fish (mean weight 0.64 g; mean total length 41 mm) were introduced into 15 L of water in each tank.
Duration:	96 hours, static test.
Test conditions:	Temperature 15 ± 1°C, pH 7.6 – 8.3. 16 hours light per day. The total hardness of dilution water was 180 mg $CaCO_3$/L. Dissolved oxygen concentrations were 80-110% of saturation over the test duration.
Observations:	Fish were monitored at 0, 24, 48, 72 and 96 hours for mortality and behavioural abnormalities. Temperature, pH and dissolved oxygen concentrations were also recorded at 24 hour intervals and water samples collected at test initiation and test termination were analysed for test substance concentration by gas chromatography.
Data analysis:	LC_{50} calculated according to Berkson et al (1953) and graphically determined.

Results:

Mean measured difenoconazole concentrations corresponded to 83-94% of nominal concentrations. Mortality data are presented in the table below.

Table B.9.2.1-10: Effect of difenoconazole on mortality in *Oncorhynchus mykiss*

Nominal concentration (mg formulation/L)	Cumulative mortality (number of dead fish)			
	24 hour	**48 hour**	**72 hour**	**96 hour**
Control	1	1	1	1
0.5	0	0	0	0
1.1	0	0	0	0
2.3	0b	0	1	1
5	0a	0a	0a	0a
11	7	7	7	7
LC_{50} (mg/L)	7.4	7.4	7.4	7.4
95% confidence interval (p=0.05)	5.6-9.8	5.6-9.8	5.6-9.8	5.6-9.8

[a] *fish suffering severe symptoms including abnormal swimming behaviour, impaired respiratory function and abnormal pigmentation*
[b] *fish showing abnormal swimming behaviour*

Based on nominal concentrations, the 96-hour LC_{50} for A-7402 H in *Oncorhynchus mykiss* was estimated to be 7.4 mg formulation/L (1.8 mg as/L).

RMS comments:

This study was performed with a formulation that is not considered to correspond to the proposed representative formulation A-7402 T. The results will therefore not be used in the further assessment.

Reference:	**Volz, E. (2004). Title: Difenoconazole 250 EC formulation (A7402T): Acute toxicity to rainbow trout (Oncorhynchus mykiss) in a 96-hour static test. [redacted] Unpublished Report No.853035. Study dates: April 15, 2004 – May 01, 2004 (Syngenta file no. 169374/2554)**
Guideline:	OECD 203 (1992), EU Commission Directive 92/69/EEC, C.1, 1992 US EPA OPPTS Test Guidelines 850.1075 (1996)
GLP:	Yes (certified laboratory).

Material and methods:

Test substance:	The formulation tested was difenoconazole 250 EC formulation (A7402T); batch no. SEZ3DP001; content of active ingredient difenoconazole (CGA169374) 252 g/L.
Species:	Rainbow trout (*Oncorhynchus mykiss)*
Treatments:	Juvenile rainbow trout, were exposed to five nominal concentrations of the formulation: 0.51, 1.1, 2.5, 5.5 and 12 mg/L.
Number of animals:	One replicate containing 7 fish were employed in the control and each test concentration.
Duration:	96 hours, static test.
Test conditions:	The total hardness of the test water was calculated to be 207 mg $CaCO_3$/L. During the study the water temperature was 13 °C. The dissolved oxygen concentrations in the test vessels ranged from 9.5 to 9.8 mg/L and pH values ranged from 8.5 to 8.7.
Observations:	The behaviour and survival of the fish were assessed at least every 24 hours after initiation of the test. Temperature, dissolved oxygen, and pH were measured at 24-hour intervals. Analytical determinations of the toxicant concentrations were made at the start and end of the exposure.
Data analysis:	Probit analysis.

Results:

The measured test concentrations (based on the analytical measurement of difenoconazole) at the start of the test ranged from 80 to 89% of the nominal values. During the test period, a decrease of the concentrations of difenoconazole in the test media was observed. The mean measured test concentrations (calculated as the average over all measurements per test concentration) varied in the range of 64 to 85% of the nominal values. The concentration of the stock solution was found to be 96% of the nominal value. All reported biological results were based on the nominal concentrations of the test compound since a formulation is tested.

The mortality of rainbow trout observed during the study is presented in the table below.

Table B.9.2.1-11: Acute toxicity of A-7402 T to rainbow trout

Concentration (mg A-7402 T /L)	Cumulative Mortality (%)			
Nominal	24h	48h	72h	96h
Control	0	0	0	0
0.51	0	0	0	0
1.1	0	0	0	0
2.5	0 *	0 *	0 *	0 *
5.5	3	7	#	#
12	7	#	#	#
LC_{50}(mg A-7402 T /L)	5.7	3.7	3.7	3.7
95% C.I.	5.48 – 6.44	3.70 – 3.72	3.70 – 3.72	3.70 – 3.72

**:symptoms of toxicity observed*
#:all fish were dead

Symptoms of toxicity observed in the fish were as follows: swimming mainly at the surface and at the bottom of aquarium, tumbling when swimming, lying on side or on back at the bottom of aquarium. The 96-hour no observed effect concentration (NOEC) value was 1.1 mg A-7402 T/L, based on observed symptoms of toxicity. The 96-hour LC_{50} of A-7402 T to rainbow trout was 3.7 (95%CL 3.70 – 3.72) mg formulation/L.

RMS comments:

Due to the decreased test concentrations during the study, the results should be based on mean measured concentrations. The notifier stated that for formulation studies it is more appropriate to calculate the results based on nominal concentrations. However, the formulation studies in this case is used to assess the risk from the active ingredient, and for precautionary reasons it is assumed that the total toxicity derives from difenoconazole, the RMS maintains that the results should be based on mean measured concentrations. This is also in line with current practice.

Due to the steep dose-response curve (from 0% to 100% mortality between two subsequent concentrations), according to OECD Guidelines 203 the data obtained are not suitable for standard methods of calculation of the LC_{50}. Further, since the dose interval is more than a factor of 2, the geometric mean approach proposed in OECD 203 is not applicable for this study. It can be concluded that the 96-hour LC_{50} of A-7402 T to rainbow trout was between 1.60 and 3.90 mg formulation/L, corresponding to 0.38 – 0.92 mg as/L. This is in line with the results from the previously described study by Voigt, 1990a, (96-hour LC_{50} 0.65 mg as/L) which will be used in the risk assessment.

B.9.2.2. Fish prolonged toxicity

ACTIVE INGREDIENT

Reference:	**Grade (1993a). Report on the prolonged toxicity test of CGA 169374 tech. To rainbow trout [redacted] Unpublished report no. 933551. (Syngenta File No 169374/0859)**
Guideline:	OECD 204 (1984).
GLP:	Yes
Material and methods:	
Test substance:	Technical difenoconazole, batch number P807002, purity 91.8%.
Species:	Rainbow trout (*Oncorhyncus mykiss*)

Treatments:	Technical difenoconazole prepared in dimethylformamide, was introduced into tanks using high precision positive displacement pumps to produce 5 nominal exposure concentrations (0.0031, 0.0125, 0.05, 0.2 and 0.8 mg/L) with a dimethylformamide concentration of 7.2 mg/L. The test incorporated one tank for each exposure concentration, one solvent control tank prepared with 7.2 mg/L dimethylformamide and one control tank.
Number of animals:	Ten fish (mean weight 1.26 g; mean length 48 mm) were introduced into each tank.
Duration:	21 days, flow-through test.
Test conditions:	Water temperature and pH ranged between 15-16°C and 7.8-8.3, respectively, over the test duration. The total hardness of dilution water ranged between 150-164 $CaCO_3$ mg/L. 16 hours light per day.
Observations:	Mortality and abnormal behaviour was monitored daily, except for Sundays. Fish weight and length was recorded on day 21. Temperature, pH, dissolved oxygen and total water hardness was recorded on alternate days. Water samples were collected on days 0, 7, 14 and 21 for analysis of test substance concentration by HPLC.
Data analysis:	Chi-square test.

Results:

With the exception of one measurement recorded in the 0.56 mg/L treatment vessel on day 16, dissolved oxygen concentrations ranged between 67 and 106% saturation. The value recorded on day 16 was 58% saturation. Although this concentration falls below the limit of 60% saturation indicated in test guidelines, the symptoms recorded for fish in this tank, appeared prior to day 16. Therefore, this deviation was not considered to affect the study results.

Overall mean difenoconazole concentrations corresponded to 40-69% of nominal concentrations. Mortality and growth rate data are presented in the tables below. Exposure to difenoconazole concentrations up to 0.023 mg/L did not have any significant adverse effect on growth rates calculated from changes in length and weight over the 21-day period. However, growth rates were significantly reduced following exposure to 0.13 and 0.56 mg/L difenoconazole.

Table B.9.2.2-1: Effect of difenoconazole on mortality in rainbow trout

Mean measured concentration (mg/L)	**Cumulative mortality (%)**			
	96 hour	**7 day**	**14 day**	**21 day**
Control	0	0	0	0
Solvent control	0	0	0	0
<0.003	0	0	0	0
0.005	0	0	0	0
0.023	0	0	0	0
0.13	0	0a	0a	0a
0.56	0b	0b	10c	10c
LC_{50} (mg/L)	>0.56	>0.56	>0.56	>0.56
95% confidence interval (p=0.05)	n.d.	n.d.	n.d.	n.d.

n.d. not determined
[a] 20-60% of fish showing abnormal pigmentation and food intake

[b] *10-50% of fish showing abnormal swimming behaviour, pigmentation and food intake*
[c] *all surviving fish showing abnormal swimming behaviour, pigmentation and food intake*

Table B.9.2.2-2: Effect of difenoconazole on growth rates in rainbow trout

Mean measured concentration (mg/L)	Growth rate based on	
	Weight	Length
Control	0.0212	0.0075
<0.003	0.0192	0.0075
0.005	0.0204	0.0074
0.023	0.0202	0.0087
0.13	0.0114	0.0050
0.56	-0.0131	0.0007

From observations of growth rate, swimming behaviour, feeding activity and pigmentation the 21-day NOEC for difenoconazole in rainbow trout were estimated to be 0.023 mg/L.

RMS comment:

The study was well performed and reported and is considered valid for the risk assessment.

METABOLITES

Reference:	**Dorgerloh M and Sommer H (2002). 1,2,4-triazole juvenile growth test, fish (*Oncohrynchus mykiss*). [redacted] Unpublished report no. DOM 21060. (Syngenta File No 71019/0052)**
Guideline:	OECD 215 (2000).
GLP:	Yes.
Material and methods:	
Test substance:	Technical CGA 71019 (1,2, 4-triazole), purity 99.9%, Batch No. NLL 7052-1.
Species:	Rainbow trout, *Oncorhynchus mykiss* (mean body length 6.1cm, mean body weight 2.4 g)
Treatments:	Nominal concentrations were 1, 3.2, 10, 32 and 100 mg CGA 71019/L. Test solutions were changed weekly and water samples were collected on days 0, 7, 14, 21 and 28 for determination of CGA 71019 concentrations.
Number of animals:	The test incorporated five replicate tanks containing ten fish for each exposure concentration and two replicate control tanks in which ten fish were exposed to untreated test water only.
Duration:	28 days, static-renewal system. The test media were changed every week.
Test conditions:	Water temperature and pH ranged between 10.8-12.8°C and 7.2-7.4, respectively. 16 hours light per day. The hardness and conductivity of dilution water were 40-60 $CaCO_3$ mg/L and <0.2 µS/cm, respectively. Dissolved oxygen concentrations were 90-103% oxygen saturation over the test duration.
Observations:	Analytical verification of the test concentrations were made in all fresh media, and on day 28 of the test period. Temperature in the test systems was measured three times a week and recorded hourly in one of the control systems. pH and dissolved oxygen were recorded weekly during the study. Fish were examined on weekdays for mortality and symptoms of toxicity. Fish were weighed on days 0 and 28 to

provide specific growth rates for each individual. Growth rate data from the pooled controls were used to estimate significant differences between the treatment groups.

Data analysis: Not stated.

Results:

The mean measured concentrations of CGA 71019 ranged from 97 to 99% of nominal values.

Body weight and growth rate data, and behaviour effects are presented in the table below. There was no mortality in control fish and only one escape-related mortality in the 1 mg/L treatment on day 9. Assuming a daily feeding rate of 3%, the mean 28-day body weight of pooled control fish was 188% of initial weight. Exposure to CGA 71019 did not have a significant effect on day 28 body weight or growth rates (r) relative to pooled control fish in the period of 0-28 days. At concentrations of ≥10mg/L, 50% of fish were inactive or displayed abnormally low activity and laboured respiration.

Table B.9.2.2-3: Effect of CGA 71019 on mean weight and growth rate in rainbow trout

Nominal concentration (mg CGA 71019 /L)	Day 0 Wet weight (g)	Day 28 Wet Weight (g)	Growth rate r over 28 days	Behaviour effects* (% of fish)
Control (1)	2.43	4.47	1.027	0
Control (2)	2.51	4.54	0.919	0
Pooled Controls	2.47	4.64	0.973	0
1.00	2.44	4.72	1.022	0
3.20	2.59	4.77	0.936	0
10.0	2.54	4.62	0.930	30
32.0	2.53	4.78	0.982	30
100.0	2.48	4.91	1.053	50

**percent of fish that were inactive or abnormally low activity, showed laboured respiration and laid on the bottom of the aquaria.*

Based on nominal concentrations and growth rate calculations, the 28-day NOEC for CGA 71019 in rainbow trout is 100 mg/L, the highest concentration tested.

RMS comments:

The study was well performed and reported. The analytical measurements of the test substance were performed in fresh media, except on day 28, where the media was 7 days old. From the day 28 measurements, however, it seems that the test compound remained stable between the exchanges of media, and therefore the use of nominal test concentrations is considered to be sufficiently reliable. Only the NOEC based on growth rate (100 mg/L) was given in the report. However, based on the behavioural effects seen at 10 mg/L and higher, the NOEC could be set to 3.2 mg/L. This value will be used in the risk assessment.

The notifier disagrees with this interpretation of the study since from their point of view the purpose of the study is to assess the effects on fish growth. According to the guideline followed (OECD 215) incidences of unusual reactions should be reported, but according to the notifier the guideline does not require an endpoint (*e.g.* NOEC) to be derived from these behavioural observations. Therefore, the notifier believes the NOEC of 100 mg CGA71019/L based on fish growth should be used in the risk assessment.

The RMS is of the opinion that also effects on behaviour could be of ecological relevance for the long term risk assessment for fish. Therefore, the NOEC value of 3.2 mg/L will be maintained.

FORMULATED PRODUCTS

SCORE 250EC

Reference:	**Voigt, H. (1991), Toxizität von CGD 96430F für Regenbogenforellen (*Salmo gairdnerii*) bei verlängerter Exposition (21 Tage).** [redacted] **Experimental phase 10 - 31 January 1991** **Syngenta File N° CGA169374/0762**
Guideline:	OECD 204 (1984).
GLP:	No but test was claimed to be conducted to sound scientific principles.
Material and methods:	
Test substance:	CGD 96430F (equivalent to A-7402 A), containing 250 g/L difenoconazole, batch no. P901011.
Species:	Rainbow trout (*Oncorhynchus mykiss*)
Treatments:	The test substance was introduced into tanks to produce nominal exposure concentrations of 0.3, 0.6, 1.0, 1.8 and 3.2 mg/L of A-7402 A.
Number of animals:	The test included one tank of 10 fish for the dilution water control and for each exposure concentration.
Duration:	21 days, semi-static test.
Test conditions:	Temperature 15.2-17.3°C, pH 7.3 – 8.5. 16 hours light per day. Dissolved oxygen concentrations ranged from 62%-88%.
Observations:	Fish were monitored daily, except weekends, for mortality and changes in behaviour. The test media were renewed every 3 to 4 days. Fish weight and length were recorded on day 21. Temperature, pH and oxygen saturation were monitored immediately prior to each medium renewal. Test substance concentrations in the lowest, an intermediate and the highest exposure concentration tanks, were analysed at the beginning and end of the first and last medium renewal intervals.
Data analysis:	Not stated.

Results:

Measurements of test substance concentrations showed that initial exposure concentrations were 80-140% of nominal, with average recoveries determined during the exposure period ranging from 86-116%. Mortality data is presented in Table B.9.2.2-5. Fish exposed to A-7402 A at 0.3 and 0.6 mg/L did not suffer mortalities or exhibit abnormal behaviour during the test. Of those fish exposed to 1.0 mg/L A-7402 A, one fish died between exposure days 1 and 4, and some of the surviving fish exhibited abnormal swimming behaviour from day 14 onwards. At 1.8mg/L, two fish died on day 20 while a further two died on day 21. Of those fish exposed to 3.2 mg/L A-7402 A, one fish died on day 11 and a further one on day 14. Almost all fish exposed to 1.8 and 3.2 mg/L exhibited symptoms of toxicity including reduced feeding, swimming at the surface or bottom,

discoloration and immobility. Fish exposed to A-7402 A at 1.8 mg/L did not feed from day 0 to day 5 and from day 14 until day 21while fish in the 3.2 mg/L treatment tank did not feed throughout the test.

Exposure to A-7402 A at concentrations up to and including 1.0 mg/L did not have any significant effects on fish body weight or length relative to control fish. Fish exposed to 1.8 and 3.2 mg A-7402 A /L had significantly lower body weight (32-44% lower) and significantly shorter body length (7-15% shorter) than that of control fish.

Table B.9.2.2-4: Measured concentrations of difenoconazole

Nominal concentration		Measured difenoconazole concentration (mg/L)			
A-7402 A (mg/L)	Difenoconazole (mg/L)	Day 0	Day 4	Day 18	Day 21
Control		<0.1	<0.1	<0.1	<0.1
0.6	0.15	0.21	0.14	0.21	0.16
1.0	0.25	0.26	0.21	0.29	0.24
3.2	0.80	0.86	0.67	0.88	0.82

Table B.9.2.2-5: Effect of A-7402 A on mortality in rainbow trout

Nominal concentration (mg/l)		Mortality (%)					
A-7402 A	Difenoconazole	Day 0	Day 4	Day 7	Day 11	Day 14	Day 21
Control	0	0	0	0	0	0	0
0.3	0.075	0	0	0	0	0	0
0.6	0.15	0	0	0	0	0	0
1.0	0.25	0	10	10	10	10	10
1.8	0.45	0	0	0	0	0	40
3.2	0.8	0	0	0	10	20	20

Based on nominal concentrations and the absence of effects on behaviour, body weight, length and mortality, the 21-day NOEC in rainbow trout was 0.6 mg/L A-7402 A (equivalent to 0.15 mg as/L).

RMS comments:

The study was reported in German. Otherwise, the study seems to be well performed and reported and is considered as valid for the risk assessment.

B.9.2.3 Fish early life stage toxicity

ACTIVE INGREDIENT

Reference:	**Surprenant, D.C. (1987b). The toxicity of CGA 169374 to fathead minnow (*Pimephales promelas*) embryo and larva. [redacted] Unpublished report no. BW-87-5-2339. (Syngenta File No 169374/0018)**
Guideline:	US EPA FIFRA 72-4.
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number 585-0812, purity 96.1%.
Species:	Fathead minnow (*Pimephales promelas*) embryo and larva.
Treatments:	Technical difenoconazole prepared in dimethylformamide, was introduced into tanks using a proportional diluter to produce 5 nominal exposure concentrations

	(0.0062, 0.012, 0.025, 0.05 and 0.1 mg/L) with a maximum dimethylformamide concentration of 0.018 mL/L. The test incorporated two replicate tanks for each exposure concentration, two solvent control tanks treated with 0.018 mL/L dimethylformamide, and two untreated control tanks.
Number of animals:	Sixty embryos were placed in embryo cups suspended in each exposure vessel and hatching was recorded daily until day 4. Forty live larvae were then selected from those surviving in each incubation cup and introduced into their respective aquaria.
Duration:	34 days, flow-through test.
Test conditions:	Mean water hardness ranged between 30-31 mg/L $CaCO_3$ over the test duration. Mean water temperatures were 24°C and mean pH varied between 6.6 and 7.2 over the test duration.
Observations:	Larvae were monitored daily for behavioural abnormalities and survival was estimated twice weekly. Larval weight and length was recorded 34 days after test initiation. Temperature, pH, dissolved oxygen and total hardness were recorded daily and water samples were collected on days 0, 1, 4 and weekly thereafter until test termination, for analysis of test substance concentration by HPLC.
Data analysis:	William's test.

Results:

Based on weekly analyses of exposure solutions, mean measured concentrations of difenoconazole corresponded to 98-123% of nominal concentrations.

Difenoconazole concentrations up to 0.1 mg/L had no significant effect on embryo survival but significantly reduced larval survival to 49%. Exposure to concentrations of 0.014, 0.029, 0.049 and 0.1 mg/L, also caused significant reductions in the length and/or wet weight of larvae after 30 days.

Table B.9.2.3-1: Effects of difenoconazole on the growth and survival of fathead minnow embryos and larvae

Mean measured concentration (mg/L)	Embryo	Larvae (30 days post hatch)		
	Survival at hatch (%)	Survival (%)	Length (mm)	Wet weight (mg)
Control	90	87	27	192
Solvent control	91	83	27	212
0.0076	92	87	26	190
0.014	62	81	26	177*
0.029	84	95	23*	136*
0.049	91	92	20*	93*
0.100	90	49*	14*	33*

**significantly different from controls ($p \leq 0.05$)*

Due to reductions in larval weight seen following exposure to 0.014 mg/L, the NOEC for difenoconazole in fathead minnows was estimated to be 0.0076 mg/L.

RMS comments:

The study was generally conducted in accordance with the referred guidelines and is considered as valid for the risk assessment. According to OECD 215, mean hardness was outside the recommended range, should be >140 mg $CaCO_3$/L. However, this is not considered to have a significant impact on the results.

Reference:	**Surprenant, D.C. (1990b). CGA 169374 technical: Toxicity to fathead minnow (*Pimephales promelas*) embryo and larva. [redacted] Unpublished report no. SLI-89-4-2961. (Syngenta File No 169374/0020)**
Guideline:	US EPA FIFRA 72-4.
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number 861408, purity 95%; ^{14}C-difenoconazole, batch number NV XXIII 64, purity 94.8%.
Species:	Fathead minnow (*Pimephales promelas*) embryo and larva.
Treatments:	Technical difenoconazole and ^{14}C-difenoconazole prepared in acetone, were introduced into tanks using a proportional diluter to produce 5 nominal exposure concentrations (0.0013, 0.0025, 0.005, 0.01 and 0.02 mg/L) with a maximum acetone concentration of 0.005 mL/L. The test incorporated two replicate tanks for each exposure concentration, two solvent control tanks treated with 0.005 mL/L acetone, and two untreated control tanks.
Number of animals:	Fifty embryos were placed in each of two embryo cups suspended in each exposure vessel.
Duration:	68 days, flow-through test.
Test conditions:	Mean water temperatures were 25°C and pH 7-7.7. 16 hours light per day. Mean total hardness of water in test aquaria ranged between 26-28 mg/L $CaCO_3$ over the test duration. Dissolved oxygen concentrations varied between and 7.2-7.7 mg/L over the test duration.
Observations:	Hatching was recorded daily until day 4 when 25 live larvae were selected from those surviving in each incubation cup and transferred to one of two larval growth cylinders in each aquaria for 60 days post-hatch exposure. Larval behaviour and mortality were monitored daily. Larval length was determined on post-hatch days 30 and 60 while and wet weight was recorded on day 60. Temperature, pH and dissolved oxygen were recorded daily while total hardness was measured on day 0 and weekly thereafter. Water samples were collected on days 0 and 4 and weekly thereafter, for analysis of test substance concentration by HPLC.
Data analysis:	Student's t-test, Bartlett's test, William's test, Dunnett's test.

Results:

Based on weekly analyses of exposure solutions, mean measured concentrations of difenoconazole corresponded to 90-160% of nominal concentrations.

Difenoconazole concentrations up to 0.019 mg/L had no significant effect on embryo survival or larvae survival measured on days 30 and 60 post-hatch. Concentrations up to 0.0087 mg/L, did not significantly affect larvae length on day 30, exposure to 0.019 mg/L difenoconazole significantly reduced length from 24 mm in controls to 23 mm. At measurement made on day 60 post-hatch, larvae length and wet weight was not significantly affected by concentrations up to 0.019 mg/L.

Table B.9.2.3-2: Effects of difenoconazole on the growth and survival of fathead minnow embryos and larvae

Mean measured concentration (mg/L)	Embryo	Day 30 post hatch		Day 60 post hatch		
	Survival at hatch (%)	Survival (%)	Length (mm)	Survival (%)	Length (mm)	Wet weight (mg)
Control	91	92	24	87	31	260
Solvent control	90	83	25	80	33	315
0.0021	92	83	24	82	32	272
0.0025	93	88	24	84	32	267
0.0045	89	82	25	79	32	287
0.0087	94	85	24	84	32	270
0.019	94	82	23*	78	32	281

**significantly different from controls ($p \leq 0.05$)*

Based on larval length measured 30 days post-hatch, the NOEC for difenoconazole in fathead minnow was 0.0087 mg/L.

RMS comments:

The study was generally conducted in accordance with the referred guidelines. According to OECD 215, mean hardness was outside the recommended range, should be >140 mg $CaCO_3$/L. However, this is not considered to have a significant impact on the results. The study is considered to be valid for the risk assessment.

B.9.2.3 Bioconcentration in fish

ACTIVE INGREDIENT

Reference:	**Forbis A.D. (1987). Uptake, depuration and bioconcentration of ^{14}C-CGA 169374 by bluegill sunfish (*Lepomis macrochirus*). [redacted] Unpublished report no. 34837. (Syngenta File No 169374/0036)**
Guideline:	US EPA FIFRA 72-6.
GLP:	Yes.
Material and methods:	
Test substance:	Unlabelled technical difenoconazole, batch number FL 851406, purity 96.1%; ^{14}C-difenoconazole, batch number CL-VII-69, specific activity 19.2 µCi/mg.
Species:	Bluegill sunfish (*Lepomis macrochirus*).
Treatments:	A proportional diluter system was used to supply a test vessel with difenoconazole, prepared in dimethylformamide, to give a nominal concentration of 0.02 mg/L and a solvent control vessel with 0.071 mL/L dimethylformamide. 48-hour equilibration period. And monitored daily for mortality and abnormal behaviour.
Number of animals:	120 fish (mean length 58 mm; mean weight 6.2 g) per test vessel
Duration:	28 days exposure, 14 days depuration. Flow through system.

Test conditions: Temperature 22±2°C, pH 8.1 – 8.3. The total hardness of dilution water was between 225-275 mg/L $CaCO_3$. Dissolved oxygen concentrations were 7.4-9.1 mg/L, respectively, over the test period.

Analysis: Fish were sampled after 4 hours, 1, 3, 7, 14, 21 and 28 days for estimation of ^{14}C content by combustion and liquid scintillation counting. During the 14 days depuration phase, fish were sampled after 1, 3, 7, 10 and 14 days for metabolite analyses. Mortality and abnormal behaviour were recorded. Temperature, pH and dissolved oxygen were recorded on the same days as fish were sampled. Water samples were also collected on these days for analysis of test substance concentration by liquid scintillation counting.

Results:

The mean water concentration of difenoconazole over the 28-day exposure period was 0.018 mg/L, i.e. 90% of nominal. However, due to malfunction of the diluter apparatus on day 27, the measured concentration on day 28 was 0.031 mg/L, i.e. 155% of nominal. Therefore day 28 data was not considered in the calculation of uptake rate, depuration rate or bioconcentration factor.

With the exception of two fish that died between days 0 and 3 of the uptake phase, all fish remained healthy throughout the study. Concentrations of ^{14}C-difenoconazole found in the tissue of fish are presented in Tables B.9.2.3-3 and B.9.2.3-4. The mean ^{14}C residue concentrations for fillet, viscera and whole fish were 5.9, 8.0 and 4.7 ppm respectively, after 28 days of continuous exposure. Analysis of fish samples taken during the depuration phase, indicated that 50% of accumulated residues were eliminated after 1.1 days in fresh water and that fish had eliminated 99, 100 and 100% of residues from fillet, viscera and whole body, respectively, by day 14. Estimated uptake rate constants, depuration rate constants and bioconcentration factors are presented in Table B.9.2.3-5.

Table B.9.2.3-3: Bioaccumulation of ^{14}C-difenoconazole in bluegill sunfish – Uptake phase

Day	Concentration of ^{14}C-difenoconazole		
	Fillet	Viscera	Whole fish
	ppm	ppm	ppm
0.17	0.53	1.5	1.0
1	1.3	3.3	2.6
3	2.1	5.3	4.1
7	2.3	8.0	5.7
14	2.3	8.7	2.7
21	2.3	8.0	4.7
28	5.9	20	13.0

Table B.9.2.3-4: Bioaccumulation of ^{14}C-difenoconazole in bluegill sunfish- Depuration phase

Day	Concentration of ^{14}C-difenoconazole					
	Fillet		Viscera		Whole fish	
	Ppm	% depuration	ppm	% depuration	ppm	% depuration
1	1.9	68	5.8	71	3.1	76
3	0.32	95	0.95	95	0.76	94
7	0.095	98	0.24	99	0.19	99
10	0.059	99	0.13	99	0.093	99
14	0.040	99	0.087	100	0.057	100

Table B.9.2.3-5: Uptake rate constants, depuration rate constants and bioconcentration factors for ^{14}C difenoconazole in bluegill sunfish.

Uptake rate constant (whole fish)	200 (±14)
Depuration rate constant	0.62 (±0.044)
Depuration half-life (days)	1.1 (±0.079)
Bioconcentration factor (mg/kg whole fish mg/L water)	320 (±32)
Time to reach 90% of steady state (days)	3.7 (±0.26)

When exposed continuously to 0.018 mg/L difenoconazole, a steady state concentration in fish tissues was reached after 3.7 days of exposure and complete depuration occurred within 14 days of transfer to clean water. The steady state bioconcentration factor for difenoconazole in bluegill sunfish was 320.

RMS comments:

The samples from day 28 could not be used for the calculations of uptake rate and bioconcentration factors, but since steady state was reached after less than four days, this would not have a significant impact on the results. No attempt was made to characterise the radioactivity, hence the BCF is based on total residues. Only one concentration was tested, while the guidelines require at least two exposure levels. Otherwise, the study was generally conducted in accordance with the referred guidelines, since a second study is available (Fackler, 1992) there is sufficient information from two different test concentrations, and therefore no further data is needed.

Reference:	**Fackler, P.H. (1992). Bioconcentration and elimination of ^{14}C-residues by bluegill (*Lepomis macrochirus*) exposed to CGA 169374. [redacted] Unpublished report no. 88-6-2737. (Syngenta File No 169374/0531)**
Guideline:	US EPA FIFRA 72-6.
GLP:	Yes.
Material and methods:	
Test substance:	^{14}C-CGA 169374, batch number BPM-V-41, specific activity 22.2 µCi/mg.
Species:	Bluegill sunfish (*Lepomis macrochirus*).
Treatments:	A proportional diluter system was used to supply a test vessel with difenoconazole, prepared in acetone, at a nominal concentration of 1.0 µg/L and a solvent control vessel with 0.007 mL/L acetone.
Number of animals:	190 fish (mean length 47 mm; mean weight 1.3 g) were added to each test vessel
Duration:	28 days exposure, 14 days depuration. Flow-through system.
Test conditions:	Mean water temperatures were 16°C and pH 6.7 – 7.1. The total hardness of dilution water used in the study was between 20-28 mg/L $CaCO_3$. Dissolved oxygen concentrations were 9-9.8 mg/L over the test duration.
Analysis:	After an equilibration period, fish were monitored daily for mortality and abnormal behaviour. Fish were sampled after 1, 3, 4, 7, 10, 14, 21 and 28 days for estimation of ^{14}C content by combustion and liquid scintillation counting. After 28 days,

remaining fish were transferred to fresh water for a further 14 days and sampled after 1, 3, 7, 10 and 14 days for metabolite analyses. Temperature was recorded daily while pH and dissolved oxygen were recorded at least three times per week for the duration of the study. Water samples were also collected on exposure days 0, 1, 3, 4, 7, 10, 11, 14, 21 and 28 and depuration days 1, 3, 7, 10, 11 and 14 for analysis of test substance concentration by liquid scintillation counting. Further, hexane/methanol extractions of muscle tissue were made to determine the relative distribution of nonpolar and polar radioactivity on day 28 of exposure.

Results:

With the exception of 8 fish that died in the difenoconazole-treated tank, all fish were healthy and exhibited normal behaviour throughout the study. The mean difenoconazole water concentration was 1.1 µg/L, i.e.110% of nominal, over the 28-day exposure period, and remained below 0.26 µg/L during the depuration phase. Concentrations of ^{14}C-difenoconazole found in the tissue of fish are presented in Table B.9.2.3-6. The mean ^{14}C residue concentrations for fillet, viscera and whole fish were 180, 610 and 340 µg/kg, respectively, after 28 days continuous exposure. Analysis of fish samples taken during the depuration phase, indicated that 50% of accumulated residues were eliminated by day 3 and that fish had eliminated 96, 98 and 97% of residues from fillet, viscera and whole body, respectively, by day 14. Estimated uptake rate constants, depuration rate constants and bioconcentration factors are presented in Table B.9.2.3-7.

Table B.9.2.3-6: Uptake and depuration of ^{14}C-difenoconazole in bluegill sunfish.

Phase	Day	Conc in water (µg/L)	Concentration of ^{14}C-difenoconazole in fish (µg/kg)		
			Fillet	Viscera	Whole fish
Uptake	1	0.93	570	640	600
	3	1.1	170	600	350
	7	1.1	180	640	330
	10	1.1	180	540	370
	14	1.2	180	600	360
	21	1.2	180	630	360
	28	1.2	180	610	340
Depuration	1	<0.26	67	270	150
	3	<0.26	33	81	53
	7	<0.21	13	26	18
	10	<0.21	10	20	14
	14	<0.21	7.2	14	10

Table B.9.2.3-7: Uptake rate constants, depuration rate constants and bioconcentration factors for ^{14}C difenoconazole in bluegill sunfish.

	Fillet	Viscera	Whole fish
Uptake rate constant	140	870	270
Depuration rate constant	0.86	1.5	0.84
Bioconcentration factor	170	570	330
Time to reach steady state (estimated by RMS)	ca 3 days	ca 3 days	ca 3 days

When exposed continuously to 1.0 µg/L difenoconazole, a steady state concentration in fish tissues was reached within 3 days of exposure and 97% depuration occurred within 14 days of transfer to clean water. The steady state bioconcentration factor for difenoconazole in bluegill sunfish was 330 based on whole fish.

Analyses of the methanol (polar) and hexane (non-polar) solvent extractions of edible tissues on day 28 revealed that 35% of the residues were extractable with methanol, 19% with hexane and 48% were not extractable with either solvent.

RMS comments:

The radioactivity in tissue samples from edible parts on day 1 was more than 3 times higher than the steady state level that was reached from day 3, and no explanation was given in the study report. A whole fish BCF calculated based on day 1 concentrations would be 645. However, in samples from day 3 onwards, the concentrations remained stable throughout the exposure phase, and therefore the proposed BCF values based on the steady state concentrations are considered reasonable. Further, the steady state BCF values are consistent with the previously referred study (**Forbis, 1987**). Also in this study, only one concentration level was tested. However, the two available studies together are considered to fulfil the requirement of more than one exposure concentration.

B.9.2.4 Acute toxicity to aquatic invertebrates

ACTIVE INGREDIENT

Reference:	**Forbis, A.D. (1988a). Acute toxicity of CGA 169374 to *Daphnia magna*. ABC Laboratories Inc., USA. Unpublished report no. 34835. (Syngenta File No 169374/0021)**
Guideline:	US EPA FIFRA 72-2.
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number FL 851406, purity 96.1%.
Species:	*Daphnia magna*
Treatments:	A stock solution of technical difenoconazole prepared in acetone, was used to produce 5 nominal exposure concentrations (0.56, 1.0, 1.8, 3.2 and 5.6 mg/L) with an acetone concentration of 0.1 mL/L. The test incorporated two replicate cultures for each exposure concentration, two solvent control vessels prepared with 0.1 mL/L acetone and two control cultures.
Number of animals:	Ten daphnids per exposure vessel (20 per test level)
Duration:	48 hours, static test.
Test conditions:	Water temperature was 20-22°C, pH 8.1-8.3. 16 hours light per day. The total hardness of dilution water used in the study was between 225-275 mg/L $CaCO_3$. Dissolved oxygen concentrations were 8.3-9.1 mg/L (>90% of saturation) over the test duration.
Observations:	The daphnids were monitored at 24 and 48 hours for mortality. Temperature, pH and

	dissolved oxygen were recorded at 0 and 48 hours. Water samples were collected at 0 and 48 hours for analysis of test substance concentration by HPLC.
Data analysis:	Binominal, moving average and probit analyses.

Results:

Overall mean measured difenoconazole concentrations corresponded to 90-100% of nominal concentrations. Mortality data are presented in the table below.

Table B.9.2.4-1: Effect of difenoconazole on mortality in *Daphnia magna*.

Mean measured concentration (mg/L)	Cumulative mortality (%)	
	24 hour	48 hour
Control	0	0
Solvent control	0	0
0.52	0	35
0.98	0	45
1.8	50	100
2.9	100	100
5.6	100	100
LC_{50} (mg/L)	1.8	0.77
95% confidence interval (p≤0.05)	0.98-2.9	0.59-0.95

Based on mean measured concentrations, the 48-hour LC_{50} for difenoconazole in *Daphnia magna* was estimated to be 0.77 mg/L.

RMS comments:

The study was conducted in accordance with the referred guidelines and is considered as valid for the risk assessment.

Reference:	**Surprenant, D.C. (1990c). CGA 169374 Acute toxicity to mysid shrimp (*Mysidopsis bahia*) under flow-through conditions. Springborn Laboratories Inc., USA. Unpublished report no. 89-2-2936. (Syngenta File No 169374/0023)**
Guideline:	US EPA FIFRA 72-3.
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number FL 861408, purity 95%.
Species:	Mysid shrimp (*Mysidopsis bahia*)
Treatments:	Technical difenoconazole prepared in acetone, was introduced into tanks using continuous flow serial diluter to produce nominal exposure concentrations of 0.036, 0.055, 0.085, 0.13 and 0.2 mg/L. The test incorporated two replicate tanks for each concentration and for each of two control treatments, prepared with and without 0.079 mg/L acetone.
Number of animals:	Ten mysid shrimp (≤ 24 hours old) per tank.
Duration:	96 hours, flow-through test.
Test conditions:	Water temperature was 23-25°C, pH 7.9-8.1. Salinity 31 – 32 promille, 16 hours light per day. Dissolved oxygen concentrations were 6.1-7.7 mg/L over the test

	period.
Observations:	Mortality was monitored daily. Temperature, pH and dissolved oxygen were recorded daily and water samples were collected on day 0 and 4 for analysis of test substance concentration by HPLC.
Data analysis:	Binominal, moving average and non-linear interpolation analyses.

Results:

Mean measured difenoconazole concentrations corresponded to 81-95% of nominal concentrations. Mortality data are presented in the table below.

Table B.9.2.4-2: Effect of difenoconazole on mortality in *Mysidopsis bahia*

Mean measured concentration (mg/L)	Cumulative mortality (%)			
	24 hour	**48 hour**	**72 hour**	**96 hour**
Control	0	0	0	0
Solvent control	0	0	0	5
0.029	0	0	0	5
0.048	0	0	0	5
0.073	0	5	5	10
0.12	30	40	45	45
0.19	30	30	60	65
LC_{50} (mg/L)	>0.19	>0.19	0.15	0.15
95% confidence interval (p≤0.05)	n.c.	n.c.	0.13-0.20	0.11-0.22

n.c. not calculated

Based on mean measured concentrations, the 96-hour LC_{50} for difenoconazole in *Mysidopsis bahia* was estimated to be 0.15 mg/L.

RMS comments:

The study was conducted in accordance with the referred guidelines, and is considered as valid for the risk assessment.

The notifier disagrees that data from the marine species, *Mysidopsis bahia* (Surprenant 1990c) may be used in place of a freshwater *Daphnia* endpoint in the tier 1 risk assessment. The EU Aquatic Guidance Document states that "At present, there is no requirement under Directive 91/414/EEC to perform these studies, but if data are available, they must be submitted and should be considered in the risk assessment. The notifier should make a reasoned case as to the relevance of data on estuarine/marine organisms to the risk assessment." In this case data are available for both oysters and mysid shrimps. These are both 96 hour studies and show toxicity endpoints of the same order as the standard 48 hour *Daphnia* test. The standard assessment factor applied at tier 1 of 100 is intended, in part, to account for interspecies sensitivity and the endpoint values for both marine species are less than one order of magnitude from the *Daphnia* value. This being the case, the notifier maintains that the risk assessment should be based on the lowest appropriate value for the standard freshwater test species, *Daphnia*. Furthermore, it should be noted that it is highly unlikely that this marine species will be exposed to residues of difenoconazole at the concentrations predicted to occur in freshwater.

From the RMS point of view, there is no reason to omit the *Mysidopsis* data from the risk assessment. Although a marine species, it represents a sensitive group that may be represented also in freshwater. There is at present no evidence that the sensitivity in general is different for salt water species compared to standard species. The current practice in the EU process is to include data from marine species in the risk assessment when studies are available.

Reference:	**Surprenant, D.C. (1990d). CGA 169374 Acute toxicity to eastern oysters (*Crassostrea virginica*) under flow-through conditions. Springborn Laboratories Inc., USA. Unpublished report no. 89-05-2988. (Syngenta File No 169374/0024)**
Guideline:	US EPA FIFRA 72-3
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number FL 861408, purity 95%.
Species:	Eastern oysters (*Crassostrea virginica*), mean valve height 40±4 mm.
Treatments:	Technical difenoconazole prepared in acetone, was introduced into tanks using a continuous flow serial diluter to produce nominal exposure concentrations of 0.044, 0.088, 0.18, 0.35 and 0.7 mg/L. The test incorporated two replicate tanks for each concentration and for each of two control treatments, prepared with and without 0.333 mL/L acetone.
Number of animals:	Ten oysters per tank.
Duration:	96 hours, flow-through test.
Test conditions:	Water temperature 19-20°C, pH 7.7-7.9. Salinity 32 – 34 promille. Dissolved oxygen concentration were 6.2-8 mg/L (77 – 99% of saturation) over the test period.
Observations:	The oysters were monitored daily for abnormalities and mortality. After 96 hours, oysters were removed for measurement of shell growth. Temperature, pH and dissolved oxygen were recorded daily and water samples were collected on day 0 and 4 for analysis of test substance concentration by HPLC.
Data analysis:	Analysis of variance and William's test.

Results:

Mean measured difenoconazole concentrations corresponded to 43-145% of nominal concentrations. Throughout the exposure period, oysters did not exhibit any abnormalities and suffer any mortality at any exposure concentration. Shell growth data, presented in the table below, indicates that shell deposition was 35% lower in oysters exposed to 0.3 mg/L difenoconazole, than in untreated oysters.

Table B.9.2.4-3: Effect of difenoconazole on shell deposition in *Crassostrea virginica*

Mean measured concentration (mg/L)	Shell deposition	
	mm (SD)	% reduction compared to the control
Control	1.7 (1.0)	-
Solvent control	1.6 (1.0)	-
0.065	1.5 (1.1)	12
0.12	1.5 (1.2)	12

0.13	1.4 (0.9)	18
0.21	1.4 (0.9)	18
0.3	1.1 (0.9)	35
EC_{50} (mg/L)	>0.3	

Exposure to difenoconazole at 0.3 mg/L caused <50% on shell deposition in *Crassostrea virginica.* Therefore the 96-hour EC_{50} was considered to be >0.3 mg/L.

RMS comments:

In the original study report, EC_{50} was reported to be 0.45 mg/L (95% CL 270 – 1400 mg/L). However, this value was extrapolated outside the range of test concentrations and is therefore considered unreliable. The RMS agrees with the value proposed by the notifier. The NOEC was reported to be 0.21 mg/L (the next highest test concentration) in the original study. However, the power of the statistical evaluation is probably low, since there was a large variation in shell deposition rate among both control and treated shells. Only the EC_{50} value (0.3 mg/L) will be used in the risk assessment.

METABOLITES

Reference:	**Bell, G. (1995). 1,2,4-triazole: acute toxicity to *Daphnia magna*. Huntingdon Life Sciences, Huntingdon, UK. Unpublished report no. ENVIR/95/52. (Syngenta File No 169374/2320)**
Guideline:	EU Directive 92/69/EEC, Method C.2; OECD 202
GLP:	Yes.
Material and methods:	
Test substance:	CGA 71019 (1, 2, 4-triazole), batch number JC 16/215854/3, purity 100.8%.
Species:	*Daphnia magna*
Treatments:	In a range-finding test, the test substance was dispersed directly into dilution water, without the use of auxillary solvents, to produce nominal concentrations of 0.1, 1, 10 and 100 mg/L. The test incorporated two vessels for each concentration level and for a control treatment of dilution water. In the definitive test, a single concentration of 100 mg/L was prepared by direct addition of the test substance to the dilution water. The test incorporated four replicate cultures for the exposure concentration and the control treatment of dilution water.
Number of animals:	Range-finding test: Ten first instar *Daphnia* per test vessel . Definitive test: Five daphnids per test vessel.
Duration:	48 hours, static test.
Test conditions:	Definitive test: Temperature 21-22°C, pH 7.6-7.9. 16 hours light per day. In the definitive test, dissolved oxygen concentrations were 7.7-8.4 mg/L over the test duration.
Observations:	**Range-finding study**: Numbers of immobilized *Daphnia* were recorded after 24 and 48 hours.

	Definitive test: Daphnids were monitored at 24 and 48 hours for immobilization. Temperature, pH and dissolved oxygen were recorded at 0 and 48 hours. Water samples were collected at 0 and 48 hours for analysis of test substance concentration.
Data analysis:	Not stated.

Results:

Measured concentrations of CGA 71019 varied from 94% of nominal at the test start to 102% of nominal at the test termination. No daphnids were immobilized in either the range-finding or definitive test. Based on nominal concentrations, the 48-hour EC_{50} for CGA 71019 in *Daphnia magna* was estimated to be >100 mg/L.

RMS comments:

The study was conducted in accordance with the referred guidelines, and is considered to be valid for the risk assessment.

Reference:	**Swarbrick, R.H. (2002). CGA 205375: Acute toxicity to *Daphnia magna*. Brixham Environmental Laboratory, UK. Unpublished report no. BL7202/B. (Syngenta File No 205375/0012)**
Guideline:	OECD 202
GLP:	Yes.
Material and methods:	
Test substance:	CGA 205375, batch number MLA-421/2, purity 99%.
Species:	*Daphnia magna*.
Treatments:	CGA 205375 was added to dilution water in exposure vessels to produce nominal exposure concentrations of 0.32, 0.56, 1.0, 1.8, 3.2 and 5.6 mg/L. The test incorporated four replicate cultures for each concentration and untreated control.
Number of animals:	Five daphnids (<24 hours old) per exposure vessel.
Duration:	48 hours, static test.
Test conditions:	Water temperature 20±1°C, pH 8-8.1. The hardness and conductivity of dilution water were 243 mg/L $CaCO_3$ and 651µS/cm, respectively. Dissolved oxygen concentrations were 8.4-9.2 mg/L (>60% of saturation) over the test period. 16 hours light per day.
Observations:	Mortality was monitored at 24 and 48 hours. Temperature was recorded daily while pH and dissolved oxygen were recorded at 0 and 48 hours. Water samples were collected at 0 and 48 hours for analysis of test substance concentration by HPLC.
Data analysis:	Moving average analysis.

Results:

Overall mean measured difenoconazole concentrations corresponded to 81-103% of nominal concentrations. Mortality data are presented in the table below.

Table B.9.2.4-4: Effect of CGA 205375 on mortality in *Daphnia magna*.

Nominal concentration (mg/L)	Cumulative immobilisation (%)	
	24 hour	48 hour
Control	0	0
0.32	0	0
0.56	0	0
1.0	0	0
1.8	70	95
3.2	95	100
5.6	95	100
LC_{50} (mg/L)	1.8	1.4
95% confidence interval (p≤0.05)	1.5-2.2	1.2-1.7

Based on nominal concentrations, the 48-hour LC_{50} for CGA 205375 in *Daphnia magna* was estimated to be 1.4 mg/L.

RMS comments:

The study was conducted in accordance with the referred guidelines, and is considered to be valid for the risk assessment.

FORMULATED PRODUCTS

DIVIDEND 030FS

Reference:	**Gries, T. (1999b). Acute toxicity test of CGA 169374 FS (30) (A-9142 G) to daphnids (*Daphnia magna*). Springborn Laboratories, Horn, Switzerland. Unpublished report no. 1047.062.110. Study dates 15-17 June 1999 (Syngenta File No. CGA 169374/1908)**
Guideline:	OECD 202
GLP:	Yes.
Material and methods:	
Test substance:	A-9142 G containing 30.6 g/L CGA 169374 (density 1049 kg/m^3). Batch number P.902001.
Species:	Daphnids (*Daphnia magna)*
Treatments:	A stock solution of test substance, prepared in water, was used to produce nominal exposure concentrations of 2.5, 5, 10, 20, 40 and 80 mg formulation/L. The test incorporated four replicate cultures for each concentration and a control treatment prepared with water.
Number of animals:	Five daphnids were introduced to each exposure vessel.
Duration:	48 hours, static test.
Test conditions:	Temperature 20±1°C, pH 7.5 – 7.6. 16 hours light per day. The hardness and conductivity of dilution water were 168 $CaCO_3$ mg/L and 445 µS/cm, respectively. Dissolved oxygen concentrations were 7.97-8.52 mg/L over the test duration.
Observations:	Cultures were monitored at 24 and 48 hours for immobilization. Water temperature of a control culture was monitored continuously while pH and dissolved oxygen concentrations were recorded at 24-hour intervals. Test substance concentrations were determined by chemical analysis of water samples taken immediately before exposure and after 48 hours.

Data analysis: Probit analysis.

Results:

Mean initial test substance concentrations corresponded to 90-114.1% of nominal concentrations.

Immobilization data are presented in the table below.

Table B.9.2.4-5: Acute toxicity of A-9142 G to *Daphnia magna*

Nominal concentration (mg/L)	Percent immobilization	
	24 hours	48 hours
Control	0	0
2.5	0	0a
5	0	0a
10	0a	0a
20	75b	90b
40	40b	65b
80	95b	100
LC_{50} (mg/L)	32	19
95% confidence interval (p=0.05)	n.d.	1.5-204

a sublethal symptoms of lethargy, floating and/or discolouration recorded in up to 50% of population
b lethargy recorded in all surviving daphids
n.d. not determined

Based on nominal concentrations, the 48-hour EC_{50} value for A-9142 G to *Daphnia magna* was estimated to be 19 mg formulation/L (0.55 mg as/L).

RMS comments:

The study was conducted in accordance with the referred guidelines. The wide confidence interval of the EC_{50} value (1.5 – 204 mg/L) was explained by the inconsistent dose-response pattern in the test. Considering the serious sublethal effects in all surviving daphnids at 40 mg/L, the RMS proposes an alternative approach for estimating the LC_{50}, based on the geometric mean of the highest concentration with no effect and the next higher concentration with 90% effect. This approach would result in an LC_{50} value of 14 mg formulation/L, or 0.43 mg as/L. These values will be used in the risk assessment for DIVIDEND 030FS.

SCORE 250EC

Reference:	**Voigt, H. (1990b), Toxizität von CGD 96430F auf Wasserflöhe (*Daphnia magna*) (48 h). Ökolimna Gesellschaft für Ökologie und Gewässerkunde mbH, Burgwedel, Germany. Unpublished Report No. 07/90/217. Experimental phase 10 - 12 July 1990** **Syngenta File N° CGA169374/0759**
Guideline:	OECD 202 I (1984).
GLP:	Yes.

Material and methods:

Test substance:	CGD 96430F (equivalent to A-7402 A) containing 250 g/L difenoconazole, batch number P901011.
Species:	*Daphnia magna* (Straus 1820), first instar at <24 hours old.
Treatments:	Nominal formulation concentrations of 0.5, 0.71, 1.00, 1.41, 2.00, 2.83, 4.00 and 5.66 mg/L A-7402 A in water.

Number of animals:	The test incorporated four replicates of five animals in 10 ml for each test concentration and the water-only control.
Duration:	48 hours, static test.
Test conditions:	Test vessels were maintained in the dark at 20±1°C. The oxygen saturation decreased from 100% at test initiation to 60% at test termination. Initial pH values were 7.4-7.5 and remained constant throughout the study.
Observations:	*Daphnia* were observed for immobilisation after 24 and 48 hours.
Data analysis:	

Results:

Test concentrations were not measured during the course of the study. Instead, measurements of a previous study were used to demonstrate that the test item remained stable in the test medium. Mean measured difenoconazole concentrations in that study corresponded to 104-118% of nominal values 2 hours after test initiation and fell to 62-100% after 96 hours. Mortality data are presented in the table below.

Table B.9.2.4-6: Effect of A-7402 A on immobilization in *Daphnia magna*

Nominal concentration (mg/L)		**% of immobilized Daphnia**	
A-7402 A	**Difenoconazole**	**24 hours**	**48 hours**
0.5	0.13	0	0
0.71	0.18	0	0
1	0.25	5	5
1.41	0.35	10	10
2	0.5	10	10
2.83	0.71	15	35
4	1	45	75
5.66	1.42	100	100
EC_{50} (mg A-7402 A)/L)		4.2	3.3

Based on nominal concentrations, the 48-hour EC_{50} of A-7402 A to *Daphnia magna* was 3.3 mg/L formulation, equivalent to 0.83 mg as/L.

RMS comments:

The study report was written in German. The lack of analytical verification of the test concentrations makes the results unreliable, and the results will not be further used in the risk assessment. However, there are no indications from other data that the SCORE 250EC formulation is more toxic than the active ingredient alone (also the results from this study indicate a similar toxicity). Further, since the risk assessment will be based on the study on *Mysidopsis*, which was more sensitive (by a factor of 5) than *Daphnia* in a short term study (Surprenant, 1990b), no further data is considered essential at this stage. Possibly, a confirmatory study may be required at MS state level.

Reference: **Grade, R. (1994a). Report on the acute toxicity of CGA 169374 EC 250 (A-7402 H) on *Daphnia (Daphnia magna* Straus 1820). Ciba-Geigy Ltd., Basel, Switzerland). Unpublished report number 943540. Experimental phase 19**

	April – 16 June 1994. Syngenta File N° CGA169374/0947
Guideline:	OECD 202.
GLP:	Yes.

Material and methods:

Test substance:	A-7402 H containing 250 g CGA 169374/L. Batch number P.308002; Density 1.0129 kg/L.
Species:	*Daphnia magna* (Straus 1820)
Treatments:	A stock solution of formulation A-7402 H, prepared in water, was used to produce nominal exposure concentrations of 0.58, 1.0, 1.8, 3.2, 5.8 and 10 mg formulation/L. The test incorporated four replicate cultures for each concentration and a control treatment prepared with reconstituted water.
Number of animals:	Five daphnids per exposure vessel.
Duration:	48 hours, static test.
Test conditions:	Temperature 20±1°C, pH 7.9 – 8.3.The total hardness of dilution water was 240 mg $CaCO_3$/L. 16 hour day-length of 1500 lux for 48 hours. Dissolved oxygen concentrations were 96 - 97% of saturation over the test duration.
Observations:	Cultures were monitored at 24 and 48 hours for immobilization. Temperature, pH and dissolved oxygen concentrations were recorded in the test systems at 0 and 48 hours, while water samples were collected at test initiation and test termination for analysis of test substance concentration by gas chromatography.
Data analysis:	Graphical determination of EC_{50}.

Results:

Mean measured difenoconazole concentrations corresponded to 69 - 81% of nominal concentrations. Mortality data are presented in the table below.

Table B.9.2.4-7: Effect of A-7402 H on immobilization in *Daphnia magna*

Mean measured concentration (mg A-7402 H/L)	Cumulative immobilization (%)	
	24 hour	**48 hour**
Control	0	0
0.44	0	0
0.76	0	15
1.46	0	5
2.36	0	10
4.50	5	55
6.91	95	100
LC_{50} (mg/L)	5.3	3.8
Confidence interval (p=0.05)	2.7-6.4	4.5-6.5

Based on mean measured concentrations, the 48-hour LC_{50} for A-7402 H in *Daphnia magna* was estimated to be 3.8 mg/L (0.938 mg as/L).

RMS comments:

The study was conducted in accordance with the referred guidelines. However, the study was performed with a formulation that is not considered to correspond to the proposed representative formulation A-7402 T. The results will therefore not be used in the further assessment.

Reference:	**Volz, E. (2004). Title: Difenoconazole 250 EC formulation (A7402T): Acute toxicity to *Daphnia magna* in a 48-hour immobilization testRCC Ltd, Environmental Chemistry & Pharmanalytics, CH-4452 Itingen / Switzerland. Unpublished Report No. 853039. Study dates: April 20, 2004 – May 01, 2004 (Syngenta file no. 169374/2552)**
Guideline:	OECD Guideline No. 202, 1984 EU Commission Directive 92/69/EEC, C.2, 1992 US EPA OPPTS Test Guidelines 850.1010, 1996
GLP:	Yes (certified laboratory).

Material and methods:

Test substance:	The formulation tested was difenoconazole 250 EC formulation (A7402T); batch no. SEZ3DP001; content of active ingredient Difenoconazole (CGA169374): 252 g/L.
Species:	*Daphnia magna*
Treatments:	Juvenile daphnids were exposed to five nominal concentrations of the formulation A-7402 T: 0.51, 1.1, 2.5, 5.5 and 12 mg/L.
Number of animals:	Four replicates containing 5 daphnids were employed in the control and each test concentration.
Duration:	48 hours, static test.
Test conditions:	The total hardness of the test water was calculated to be 250 mg $CaCO_3$/L. During the study the water temperature was maintained at 19 to 20 °C. The dissolved oxygen concentrations in the test vessels ranged from 8.6 to 9.3 mg/L and pH value ranged from 7.9 to 8.0.
Observations:	The immobility was assessed after 24 and 48 hours after initiation of the test. Temperature, dissolved oxygen, and pH were measured at 24-hour intervals. Analytical determinations of the toxicant concentrations were made at the start and end of the experiment.
Data analysis:	Probit analysis.

Results:

The analytically determined test item concentrations in the analyzed test media from the start and the end of the test varied in the range from 81 to 91% of the nominal values. In the stock solution sample 98% of the nominal concentration was measured. The mean measured concentrations (calculated as the average over all measurements per test concentration) ranged from 83 to 90% of nominal. All reported biological results are based on the nominal concentrations. The immobilisation of the daphnids observed after 24 and 48 hours test duration is presented in the table below.

Table B.9.2.4-8: Acute toxicity of A-7402 T to *Daphnia magna*

Concentration (mg A-7402 T /L)	**Immobility (%)**	
Nominal	24h	48h
Control	0	0
0.51	0	0
1.1	0	0
2.5	0	0
5.5	90	95
12	100	100
EC_{50} (mg A7402T /L)	4.5	4.3
95% C.I.	n.d.	n.d.

n.d.:not determined

The 48-hour no observed effect concentration (NOEC) value was 2.5 mg A-7402 T /L, based on observed immobility. The 48-hour EC_{50} of A-7402 T to *Daphnia magna* was determined to be 4.3 mg/L.

RMS comments:

Due to the steep dose-response curve (from 0% to 95% mortality between two subsequent concentrations), according to OECD Guidelines 203 the data obtained are not suitable for standard methods of calculation of the LC_{50}. Further, since the dose interval is more than a factor of 2, the geometric mean approach proposed in OECD 203 is not applicable for this study. It can be concluded that the 48-hour EC_{50} of A-7402 T to *Daphnia magna* was between 2.5 and 5.5 mg formulation/L, corresponding to 0.62 – 1.38 mg as/L. This is more or less in line with the results from the previously described study on the active ingredient by **Forbis 1988a (**48-hour EC_{50} 0.77 (95%CL 0.59 – 0.95 mg as/L). The study is considered to be of sufficient quality to fulfil the requirement of data on the representative formulation SCORE 250EC.

B.9.2.5 Chronic toxicity to aquatic invertebrates: *Daphnia magna*

ACTIVE INGREDIENT

Reference:	**Forbis, A.D. (1988b). Chronic toxicity of CGA 169374 to *Daphnia magna* under flow-through test conditions. ABC Laboratories Inc., USA Unpublished report no. 34836. (Syngenta File No 169374/0022)**
Guideline:	US EPA FIFRA 72-4.
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number FL 851406, purity 96.1%.
Species:	*Daphnia magna*
Treatments:	Technical difenoconazole prepared in acetone, was introduced into exposure vessels, using an intermittent-flow proportional diluter system, to produce nominal exposure concentrations of 0.0036, 0.006, 0.012, 0.022 and 0.05 mg/L. The test incorporated four replicate cultures for each concentration and each of two control treatments, prepared with and without 0.05 mL/L acetone.
Number of animals:	Ten daphnids per exposure vessel.
Duration:	21 days, flow-through test.

Test conditions:	The hardness and conductivity of dilution water were 206-275 mg/L $CaCO_3$ and 500-650 µmhos/cm, respectively. Dissolved oxygen concentration were 7 - 8.9 mg/L over the test period. Water temperature 20°C, pH 8.1-8.3.
Observations:	The daphnids were monitored daily for mortality and reproductive success. Temperature, pH and dissolved oxygen were recorded on days 0, 4, 7, 14 and 21. Water samples were collected on the same days for analysis of test substance concentration by HPLC.
Data analysis:	Analysis of variance, Dunnett's test.

Results:

Mean measured difenoconazole concentrations corresponded to 93-108% of nominal concentrations.

The effects on adult mortality, reproduction and growth are shown in the table below. Exposure to difenoconazole concentrations up to 0.053 mg/L did not have any significant effect on mortality. However, adult length measured on day 21 and mean number of young per adult per reproduction day, were significantly reduced by 5-13% and 34-79%, respectively, at mean measured difenoconazole concentrations of 0.013, 0.021 and 0.053 mg/L.

Table B.9.2.5-1: Effects of difenoconazole on *Daphnia magna* mortality, reproduction and growth

Mean measured concentration (mg/L)	Mean percent survival (n=40)	Mean young per adult per reproduction day	Mean adult length Day 21 (mm)
Control	85	7.9	4.0
Solvent control	95	8.0	3.9
Pooled controls	90	8.0	4.0
0.0036	100	8.1	3.9
0.0056	90	7.2	3.9
0.013	100	6.3*	3.8*
0.021	98	5.4*	3.8*
0.053	98	2.7*	3.6*

** significantly different to pooled (solvent and untreated) controls ($p \leq 0.05$). One-way analysis of variance and Dunett's test.Variation within groups was small.*

Based on effects on mean young per adult per reproduction day and mean adult length, the 21-day NOEC for difenoconazole in *Daphnia magna* was 0.0056 mg/L (mean measured).

RMS comments:

The study was conducted in accordance with the referred guidelines (US EPA 74-2). In the corresponding OECD guidelines (202), also the time to first brood is proposed as a relevant observation endpoint. Looking at the reproduction data reported for each test vessel at different time points during the study, a delay of time to first brood was indicated at 0.0056 mg/L and higher concentrations – on day 7, the number of instars were lower than 50% of that in the control at 0.0056 mg/L, and at the two highest concentrations no instars were observed until day 12. Based on this possible effect on time to first brood, the 21-day NOEC for difenoconazole in *Daphnia magna* would be 0.0036 mg/L (mean measured). However, it should be noted that broods were not assessed between days 7 and 12, and therefore it is not possible to fully assess the magnitude of the delay to first brood from this study. Further, based on off-spring data from later sampling days there is no apparent difference

compared to the control and hence there seems to be a recovery of the reproduction potential from day 12 onwards at 0.0056 mg/L.

There also seemed to be a dose related effect on the cumulative number of young over 21 days at 0.0056 mg/L and higher (see table below). However, the data analysis reported indicated that this was not statistically significant.

Table B.9.2.5-2: Mean number of instar per test vessel during the 21 day study (n=4).

Mean measured concentrations	Control	Solvent control	0.0036 mg/L	0.0056 mg/L	0.013 mg/L	0.021 mg/L	0.053 mg/L
Day 7	136	130	127	61.3	31.8	0	0
Day 12	338	365	378	364	271	288	105
Day 14	37.8	31.8	84.5	57.0	59.3	65.8	149
Day 16	232	287	253	217	242	159.8	46.3
Day 19	171	177	151	168	161	100.8	0
Day 21	79	41.5	61.8	47	58.3	90	50.5
Cumulative number over 21 days	993.8	1032.3	1055.3	914.3	823.4	704.4	350.8
Cumulative number over 21 days (% of pooled control)	-	-	104	90	81	70	35

In conclusion, the NOEC should be based on the number of young per adult, and was determined to be 0.0056 mg/L, as proposed by the study author. This value will be used in the risk assessment.

Reference:	**van der Kolk, J (1999). CGA 169374: Chronic effects on midge larvae (*Chironomus riparius*) in a water/sediment system. Springborn Laboratories AG, Switzerland. Unpublished report no. 97-192-1008. (Syngenta File No 169374/1816)**
Guideline:	ASTM E1706 (1995).
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number P.807002, purity 91%.
Species:	Larvae of *Chironomus riparius* (syn. *Chironomus thummi*), 2-3 days old.
Treatments:	Nominal concentrations 0.05, 0.5, 5 and 50 mg/kg dry sediment. Artificial sediment (according to OECD 207) containing technical difenoconazole mixed with quartz sand, was transferred to test chambers and overlaid with Elendt M4 medium to a depth of 16.5 cm. A control treatment was prepared by mixing quartz sand with artificial sediment. The systems were equilibrated for 24 hours prior to the test.
Number of animals:	25 midges per chamber, two replicates per treatment level.
Duration:	28 days, static system.
Test conditions:	Temperature 20±2°C, water pH 7.7, sediment pH 5.9. Organic carbon content of the sediment was not reported. The hardness and conductivity of dilution water were 240 mg/L $CaCO_3$ and 610 µS/cm, respectively. The larvae were fed with Tetramin fish-food during the test.

Observations: Cultures were monitored daily for numbers of emerged midge. Water samples were collected on day 21 for analysis of test substance concentration by HPLC.

Data analysis: Analysis of variance.

Results:

Chironomid emergence occurred between days 13 and 22 of the study, Exposure to difenoconazole concentrations up to 50 mg/kg dw did not significantly affect mean emergence rates or mean chironomid development rates.

Table B.9.2.5-3: Effect of difenoconazole on emergence and development of *Chironomus riparius*.

Nominal concentration (mg/kg dw sediment)	Mean emergence (% of initial larvae)	Mean development rate (proportion of total emerged midges emerging per day)
Control + quartz sand	80	0.067
0.05	72	0.069
0.5	66	0.066
5	78	0.067
50	70	0.064

Based on absence of effects on emergence or development rate in *Chironomus riparius* at concentrations of 0.05-50 mg/kg dw, the 28-day NOEC for difenoconazole was considered to be 50 mg/kg sediment, the highest test concentration.

RMS comments:

Due to the lack of analytical measurements in the sediment phase, the NOEC based on sediment concentration should also be treated with caution. However, since no effects were observed in the study, and since other aquatic invertebrates are indicated to be much more sensitive to difenoconazole, this study is considered to be of sufficient quality for the assessment of the risk for sediment-dwelling organisms. NOEC based on the measured concentration in the water phase on day 21 was 0.015 mg as/L.

METABOLITES

Notifier: Due to the low acute toxicity of CGA 205375 and CGA 71019 to *Daphnia magna*, chronic toxicity tests were not considered necessary and therefore not submitted.

RMS: The argument provided by the notifier is considered to be consistent with the recommendations in the Aquatic Guidance Document. Since the metabolite CGA 71019 was more than a factor of 100 less toxic than the parent in the acute studies, no chronic data is needed. With regard to the other major metabolite, CGA 205375, acute toxicity to fish was slightly higher than that for the parent. However, CGA 205375 is mostly formed in and associated to the sediment compartment and therefore, the chronic toxicity of CGA 205375 can be considered to be covered by the study on *Chironomus* (Grade, 2001, summarised below). In addition, since the risk assessment of the parent is driven by aquatic invertebrates which were slightly more sensitive than fish (lowest NOEC 0.0056 mg/L for invertebrates, 0.0076 mg/L for fish) in the long term studies, possible risks to fish from CGA 205375 is considered to be covered by the present data.

Reference:	**Grade, R. (2001). Toxicity test of CGA 211391 (metabolite of CGA 169374) on sediment-dwelling *Chironomus riparius* (syn. *Chironomus thummi)* under static conditions. Syngenta Crop Protection AG, Switzerland. Unpublished report no. 2003511. (Syngenta File No 211391/0001).**
Guideline:	OECD proposed guideline for toxicity test with Chironomidae, May 1998; BBA Guideline proposal 1995.
GLP:	Yes.

Material and methods:

Test substance:	CGA 205375 (synonymous with CGA 2113910, batch number RL-2383/4, purity 99%.
Species:	Larvae of *Chironomus riparius* (syn. *Chironomus thummi*), 2-3 days old.
Treatments:	Nominal concentrations added to the water column: 0.025, 0.05, 0.1, 0.2, 0.4 and 0.8 mg/L or mixed with sediment: 2.5, 5, 10, 20, 40 and 80 mg/kg dw sediment. Artificial sediment, with or without technical difenoconazole, was transferred to test chambers and overlaid with M4 medium to a depth of 8 cm. The test incorporated three replicate cultures for each concentration and untreated controls. Establishment period of 9 days for the water application and 3 days for the sediment application before addition of the test larvae. For water applications, aliquots of a difenoconazole stock solution were introduced into the water column 1 day later.
Number of animals:	20 midge larvae per chamber.
Duration:	26 days in the case of water applications, and 28 days for sediment applications. Static systems.
Test conditions:	Temperature 20.8-21.5°C, pH 7.9-10.2. Water hardness and conductivity were 250-300 mg/L $CaCO_3$ and 710-807 µS/cm, respectively. Dissolved oxygen concentrations were 6.2-9.5 mg/L. The larvae were fed with fish-food during the test.
Observations:	Cultures were monitored daily for numbers of emerged midge. Temperature, pH and dissolved oxygen concentration were recorded once a week. Water samples were collected from vessel on days 0, 2, 7, 14 and 26/28 for analysis of test substance concentration by HPLC. Sediment concentrations were determined in samples collected on days 0, 7 and 26/28.
Data analysis:	Dunnett's test, logit analysis.

Results:

Measured concentrations of CGA 205375 in water samples from cultures exposed via the water column were 0.02, 0.05, 0.1, 0.19, 0.38 and 0.81 mg/L corresponding to 80-101.3% of nominal concentrations. Measured concentrations of CGA 211391 in water samples from cultures exposed via the sediment were below the limits of detection for 2.5 and 5 mg/kg dw doses and 0.02, 0.03, 0.07, 0.15 and 0.33 mg/L for 10, 20, 40 and 80 mg/kg dw doses.

CGA 205375 concentrations up to 0.8 mg/L applied to the water column and 10 mg/kg dw applied to sediment did not significantly affect chironomid emergence rates. Exposure to water column concentrations of 0.8 mg/L and sediment concentrations of 20, 40 or 80 mg/kg dw significantly reduced emergence by up to 100%.

Table B.9.2.5-4: Effect of water column applications of CGA 205375 on the emergence and development of *Chironomus riparius*

Nominal concentration (mg/L)	Mean emergence (% of initial larvae)	Mean development rate (proportion of total emerged midges emerging per day)
Control	76	0.0556
0.025	83	0.0570
0.05	72	0.0610
0.1	72	0.0574
0.2	72	0.0514
0.4	68	0.0500
0.8	8*	n.c.
NOEC (mg/L)	0.4	0.4
EC_{50} (mg/L)	0.5	1.5**
95% confidence interval (p≤0.05)	n.c.	n.c.

**significantly different from control (p≤0.05)*
***extraplolated value*
n.c. not calculated

Table B.9.2.5-5: Effect of sediment applications of CGA 205375 on the emergence and development of *Chironomus riparius.*

Nominal concentration (mg/kg dw sediment)	Mean emergence (% of initial larvae)	Mean development rate (proportion of total emerged midges emerging per day)
Control	92	0.0649
2.5	90	0.0697
5	95	0.0682
10	83	0.0658
20	5*	0.0672
40	0*	n.c.
80	0*	n.c.
NOEC (mg/kg dw)	10	40
EC_{50} (mg/kg dw)	13.9	551**
Confidence interval (p≤0.05)	11.3-16.2	n.c.

**significantly different from control (p≤0.05)*
***extrapolated value*
n.c. not calculated

Based on effects on emergence or development rate in *Chironomus riparius* the 26-day NOEC for exposure via the water column was 0.4 mg/L and the 28-day NOEC for exposure via sediment was 10 mg/kg.

RMS comments:

The study was well performed and reported, although the extrapolated EC_{50} values should be treated with caution. No degradation of the test substance took place during the study. The NOEC values are considered valid for the risk assessment.

FORMULATED PRODUCTS

SCORE 250 EC

Reference:	**Voigt, H. (1990c), Toxizität von CGD 96430F für Wasserflöhe (*Daphnia magna*) (21 Tage). Ökolimna Gesellschaft für Ökologie und Gewässerkunde mbH, Burgwedel, Germany. Unpublished Report No. 07/90/218, 09 October 1990. Experimental phase 01 - 22 August 1990 Syngenta File N° CGA169374/0760**
Guideline:	OECD 202 II (1984).
GLP:	Yes.

Material and methods:

Test substance:	CGD 96430F (equivalent to A-7402 A) containing 250g/L difenoconazole, batch no. P 901011.
Species:	*Daphnia magna*.
Treatments:	Nominal concentrations of the formulation A-7402 A corresponded to active ingredient concentrations of 0.072, 0.179, 0.448, 1.120, 2.80 and 7.00 mg as/L.
Number of animals:	Four replicates of ten animals for each test concentration and the water-only control.
Duration:	21 days, semi-static test. The test media were renewed every 3 to 4 days.
Test conditions:	During the study, water temperature was maintained at 21 ± 1°C. pH values were 7.8-8.2 over the test period. Oxygen saturation changed from 100% at test initiation to 68-103% immediately prior to medium renewal.
Observations:	Temperature, pH and oxygen saturation were recorded. Mortality, immobility, behaviour and effects on reproduction were recorded during the study. No analytical measurements were performed in this study.
Data analysis:	Probit analysis.

Results:

Test substance concentrations were not measured during the course of the study. Instead, the notifier proposed that measurements from a previous study could be used to demonstrate that the test substance remained stable in the test medium (82 – 100% of the nominal values). The effects of A-7402 A on immobilization and reproduction are shown in the tables below.

Table B.9.2.5-6: Effect of A-7402 A on immobilization in *Daphnia magna*

Nominal concentration (mg/L)		**Immobilisation (%)**			
Formulation	**Difenoconazole**	**Day 2**	**Day 5**	**Day 14**	**Day 21**
Control	-	0.0	0.0	0.0	0.0
0.072	0.018	0.0	0.0	2.5	5.0
0.179	0.045	2.5	2.5	7.5	12.5
0.448	0.112	35.0	35.0	40.0	47.5
1.120	0.280	45.0	95.0	100.0	100.0
2.800	0.700	55.0	100.0	100.0	100.0
7.000	1.750	100.0	100.0	100.0	100.0
EC_{50} (mg as/L)		0.45	0.13	0.12	0.12

Table B.9.2.5-7: Effect of A-7402 A on reproduction in *Daphnia magna*

Nominal concentration (mg/L)		Number of offspring per day							
Formulation	**Difenoconazole**	**Day 8**	**Day 9**	**Day 12**	**Day 13**	**Day 14**	**Day 16**	**Day 19**	**Day 21**
Control	0	223	66	414	71	282	216	412	566
0.072	0.018	23	226	353	79	98	584	573	206
0.179	0.045	0	54	262	254	99	255	293	222
0.448	0.112	0	0	18	0	0	100	43	0
1.120	0.280	0	-	-	-	-	-	-	-
2.800	0.700	-	-	-	-	-	-	-	-
7.000	1.750	-	-	-	-	-	-	-	-

Based on nominal concentrations, the 21-day NOEC of A-7402 A in *Daphnia magna* was 0.072 mg/L formulation, equivalent to 0.018 mg as/L.

RMS comments:

The study report was written in German. The lack of analytical verifications of the test concentrations makes the results unreliable, and the results will not be used in the further assessment. According to the guidance document on aquatic ecotoxicology (SANCO 8075/VI/97) a long term study with the formulation is not deemed necessary when the formulation is less than one order of magnitude higher more toxic than the active ingredient alone in short term studies. Hence, no further data is considered necessary for this formulation.

Reference:	**Neumann, C. (1997). Acute toxicity test of CGA 169374 EC 250 (A-7402 G) on sediment-dwelling larvae of *Chironomus riparius* (syn. *Chironomus thummi*) under static conditions. Novartis Crop Protection, Basel, Switzerland. Unpublished report number 953501. Experimental phase 23 March – 13 July 1995.** **Syngenta File N° CGA169374/1359**
Guideline:	BBA/IVA ring-test protocol (1994).
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 G containing 255 g CGA169374/L; Batch number P.210005; Density 1.0676 g/cm^3
Species:	Sediment-dwelling larvae of *Chironomus riparius* (syn. *Chironomus thummi*)
Treatments:	Test chambers containing artificial sediment to a depth of 2-3cm, overlaid with Elendt M4 medium to a depth of 18 cm were prepared 5 weeks prior to test initiation. Chironomid larvae were introduced into each vessel 1 day prior to test substance application. Nominal concentrations of 0.008, 0.22, 0.48, 1.05, 2.3 and 5 mg formulation/L. The test incorporated two replicate cultures for each concentration and a control treatment.
Number of animals:	25 larvae per test vessel.
Duration:	28 days, static test.
Test conditions:	Temperature 20 ± 2°C, pH 8.4-9.9. 16 hours light per day (1100 Lux). Water hardness and conductivity were 244 mg $CaCO_3$/L and 633 µS/cm, respectively. Dissolved oxygen concentrations were 5.8-9 mg/L. The larvae were fed with fish-food during the test.

Observations:	Visual assessments of behaviour, mortalities and emergence were made daily except on days 12, 16, 24, 25 and 28. On emergence, midge sex and length were recorded. Temperature, pH and dissolved oxygen concentrations were recorded on day 0, 7, 20 and 30. Water samples were collected on days 0, 2, 8, 20 and 30 and at test termination for analysis of test substance concentration by gas chromatography.
Data analysis:	Dunnett's or William's test, probit analysis.

Results:

Analysis of water samples taken on day 0 indicated that initial mean measured concentrations of A-7402 G were 53-77% of nominal, respectively.

Emergence and midge length data are presented in Table B.9.2.5-8. In untreated vessels, emergence began on day 13 and was complete by day 17 when 84% of introduced larvae had emerged. A-7402 G concentrations up to 1.05 mg/L did not have any effect on emergence rate or the time of emergence. Although emergence rate was not affected following exposure to 2.3 mg/L, emergence was delayed with the majority of midges emerging between days 14 and 18. Emergence from larvae exposed to 5 mg/L was significantly reduced to 38% and delayed to between days 21 and 29. Midge length was not affected by exposure to A-7402 G concentrations up to 5 mg/L.

Regression analysis of development rate parameters calculated from emergence data produced an EC_{50} of 4.3 mg A-7402 G/L (1.03mg difenoconazole/L). EC_{50} for emergence rate could not be estimated by regression analysis.

Table B.9.2.5-8: Effect of difenoconazole on emergence and midge length of *Chironomus riparius*

Nominal concentration (mg/L)	Actual concentration day 0 (mg/L)	Total emerged midges (% of initial; emergence rate)	Mean midge length at test termination (mm)	
			Male	Female
Control	-	84	6546	6418
0.008	0.0042	92	6591	6608
0.22	0.15	88	6522	6572
0.48	0.35	84	6734	6614
1.05	0.7	92	6744	6578
2.3	1.75	108	6832	6836
5	3.85	38	6648	6573

The NOEC values for *Chironomus riparius,* based on emergence and development rates (and measured concentrations on day 0) were 1.75 and 0.7 mg A-7402 G/L, (0.42 and 0.17 mg difenoconazole/L), respectively.

RMS comments:

The presented results were based on the measured concentrations on day 0. Since the concentrations were significantly decreased during the study, this approach may underestimate the toxicity. From the concentrations in samples at different timepoints (days 0, 8, 20 and 30) during the study, the DT_{50} in the water phase can be roughly estimated to ca one week. In this study, the mean measured concentrations were 0.0024, 0.043, 0.14, 0.30, 0.73 and 1.9 mg formulation/L, respectively, resulting in a NOEC of 0.30 mg formulation/L (or 0.075 mg as/L) based on development rate. This value will be used in the risk assessment.

The notifier stated that for formulation studies it is more appropriate to calculate the results based on nominal concentrations. However, the formulation studies in this case is used to assess the risk from the active ingredient, and for precautionary reasons it is assumed that the total toxicity derives from difenoconazole, the RMS maintains that the results should be based on mean measured concentrations. This is also in line with current practice.

B.9.2.6 Effects on algal growth and growth rate

ACTIVE INGREDIENT

Reference:	**Rufli, H. (1989). Algal growth inhibition test of CGA 169374 to green algae (*Scenedesmus subspicatus*). Ciba Geigy Ltd., Basel Switzerland. Unpublished report no. 881699. (Syngenta File No 169374/0026)**
Guideline:	OECD 201 (1984).
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number FL 807002, purity 91.8%.
Species:	Green algae (*Scenedesmus subspicatus*).
Treatments:	Nominal concentrations (0.63, 1.3, 2.5, 5.0, 10 mg/L) of technical difenoconazole.
Initial cell density:	1.3 x 10^4 cells/mL. The test incorporated three replicate cultures for each exposure concentration and six replicates of an untreated control.
Duration:	72 hours, static test.
Test conditions:	Temperature 23±2°C, pH 7.2 – 9.3. Continuous light of 8000 lux.
Observations:	Cultures were assessed daily using a cell counter and water samples were collected at 0 and 72 hours for analysis of test substance concentration by GLC. Water pH was recorded at 0 and 72 hours.
Data analysis:	Graphical determination of EC_{50} values. Dunnett's test for determination of NOEC.

Results:

Mean initial difenoconazole concentrations corresponded to 117-148% of nominal concentrations. Mean cell densities and inhibition values are presented in the table below.

Table B.9.2.6-1: Effect of difenoconazole on algal cell density.

Nominal concentration (mg/L)	Mean measured concentration (mg/L)	Mean cell density (cells/mL*10000)			Mean inhibition (%) (area under the growth curve)
		24 hour	48 hour	72 hour	0-72 hour
Control	-	6.1	38.7	145.8	0
0.63	0.87	5.3	29.2	100.7	28.8
1.3	1.7	3.8	14.3	37.7	70.5
2.5	3.2	3.2	7.2	13.5	87.8
5	6.9	2.5	3.4	5.8	95.1
10	12	2.4	3.3	3.7	96.3

Based on mean measured concentrations, the 72-hour E_bC_{50} for difenoconazole in *Scenedesmus subspicatus* was estimated to be 1.2 mg/L.

RMS comments:

The study was conducted generally in accordance with the referred guidelines. A pH increase from 7.5 to 9.3 (1.8 units) were observed in the control, but in the treated samples the pH remained within the recommended range (1.0 units). No confidence intervals were given for the EC_{50} value. The EC_{50} value reported in the study was based on biomass only (area under the growth curve), therefore the RMS has made an assessment also of the effects on growth rate.

Table B.9.2.6-2: Effect of difenoconazole on algal growth rate.

Mean measured conc (mg/L)	Initial cell density (cells/mL)	Mean cell density 72 h (cells/mL)	Growth rate (0-72 h)	Mean inhibition (%) (growth rate 0-72 h)
control	13000	1458000	0.0656	100
0.87	13000	1007000	0.0604	92.2
1.7	13000	377000	0.0468	71.3
3.2	13000	135000	0.0325	49.6
6.9	13000	58000	0.0208	31.7
12	13000	37000	0.0145	22.2

The probitanalys based on growth rate data resulted in an EC_{50} of 3.8 (95%CL 3.3-4.5) mg as/L. This value will be used as a supplement in the risk assessment.

Reference:	**Grade, R. (1993b). Report on the growth inhibition test of CGA 169374 tech. to green algae (*Scenedesmus subspicatus*). Ciba Geigy Ltd., Basel Switzerland. Unpublished report no. 938153. (Syngenta File No 169374/0860)**
Guideline:	OECD 201 (1984).
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number P.807002, purity 91.8%.
Species:	Green algae (*Scenedesmus subspicatus*).
Treatments:	Nominal concentration were 0.0123, 0.037, 0.11, 0.33 and 1.0 mg/L. Cultures (Cambridge 276/20) were treated with aliquots of a stock solution containing difenoconazole prepared in water with 0.04 mg/L Tween 80. The test incorporated three replicate cultures for each exposure concentration, six replicates of a solvent control prepared with Tween 80 (0.04 mg/L) and six replicates of an untreated control.
Initial cell density:	9900 cells/mL.
Duration:	72 hours, static test.
Test conditions:	Temperature 24±1°C, pH 7.7 – 8.1. Continuous light of 8000 lux.
Observations:	Cell density was assessed daily using a cell counter. Samples of culture solutions were taken immediately prior to exposure and after 72 hours for analysis of test substance concentrations by GLC.

Data analysis: Maximum likelihood method, logit model.

Results:

GLC analysis showed that mean initial difenoconazole concentrations for nominal concentrations of 0.0123 and 0.037 mg/L were below the limits of detection (<0.04 mg/L) while mean initial concentrations for nominal concentrations of 0.11, 0.33 and 1.0 mg/L were 77, 65 and 70% of nominal. Mean cell densities and inhibition values are presented in the table below.

Table B.9.2.6-3: Effect of difenoconazole on algal cell density.

Nominal concentration (mg/L)	Mean measured concentration (mg/L)	Mean cell density (cells/mL*10000)			Mean inhibition (%)
		24 hour	48 hour	72 hour	0-72 hour
Control	n.a.	3.6	19.2	113.3	0
Solvent control	n.a.	3.9	20.1	118.0	0
0.0123	<0.04	3.8	17.1	112.8	4.1
0.037	<0.04	3.4	14.3	63.3	39.9
0.11	0.085	3.1	4.5	7.0	89.0
0.33	0.22	2.4	1.7	4.7	95.0
1.0	0.72	1.6	1.1	1.8	98.6

Based on mean measured values for those concentrations above the limit of detection and assuming the actual concentration of those doses below the limit of detection were 70% of nominal, 72 hour E_bC_{50} and NOEC parameters were estimated to be 0.032 (95% confidence interval ($p \leq 0.05$) of 0.026 to 0.039 mg/L), and 0.0086 mg/L, respectively.

RMS comments:

The analytical measurements at the concentrations close to the EC_{50} and NOEC values were below the limit of detection. However, the proposed assumption that the real concentrations are 70% of the nominal values seems as a reasonable worst case approach. This value was supported by measured concentrations at higher levels, and furthermore from other aquatic studies it seems that the maintenance of the test concentrations is better at lower concentrations (probably related to solubility).

The EC_{50} value reported in the study was based on biomass only (area under the growth curve), therefore the RMS has made an assessment also of the effects on growth rate.

Table B.9.2.6-4: Effect of difenoconazole on algal growth rate.

Mean measured conc (mg/L)	Initial cell density (cells/mL)	Mean cell density 72 h (cells/mL)	Growth rate (0-72 h)	Mean inhibition (%) (growth rate 0-72 h)
control	9900	1133000	0.0658	100
solvent	9900	1180000	0.0664	101
0.00861	9900	1128000	0.0658	99.9
0.0259	9900	633000	0.0577	87.7
0.085	9900	70000	0.0272	41.3
0.22	9900	47000	0.0216	32.9
0.7	9900	18000	0.0083	12.6

It was concluded that the growth rate data did not fit to the probit model, and hence no E_rC_{50} could be calculated. However, visual assessment of the data indicates that the biomass data should be protective enough.

METABOLITES

Reference:	**Palmer SJ, Kendall TZ and Krueger HO (2001). 1,2,4-triazole: A 96-hours toxicity test with the freshwater alga (*Selenastrum capricornutum*). Wildlife International Ltd, USA. Unpublished report no. 528A-101. (Syngenta File No 71019/0044)**
Guideline:	OECD 201; EU Directive 92/69/EEC, Method C.3; U.S. EPA OPPTS Number 850.5400
GLP:	Yes.
Material and methods:	
Test substance:	Technical CGA 71019 Batch no: EN 38530; Purity 99%.
Species:	Green algae (*Selenastrum capricornutum/Pseudokirchneriella subcapitata*).
Treatments:	Three replicate cultures were exposed to nominal concentrations of 1.9, 3.8, 7.5, 15 and 30 mg CGA 71019/L and a negative (culture medium) control. One additional replicate was maintained in each control and treatment group to provide test solution for verification of the test substance concentration.
Initial cell density:	1 x 10^4 cells/mL.
Duration:	96 hours, static test.
Test conditions:	Temperature 23±2°C, pH 8.0 – 9.7. Continuous cool-white fluorescent light, 6500 Lux±10%.
Observations:	Test substance concentrations were measured at 72 and 96 hours. pH was measured at 0 and 96 hours. Algal samples were collected from each test culture at 24-hour intervals to determine cell densities. Cell densities, areas under the growth curve (biomass) and growth rates were used to calculate percent inhibition values relative to the control.
Data analysis:	Shapiro-Wilk's test and Levene's test. Dunnett's test.

Results:

Concentrations of CGA 71019 ranged from 86% to 101% of nominal at 0 hours and from 78% to 103% of nominal at 96 hours. Mean cell densities and percent inhibition values are presented in the tables below.

Table B.9.2.6-5: Effect of CGA 71019 on algal cell density

Mean measured concentration (mg/L)	Mean cell density (% inhibition relative to control)			
	24 hours	**48 hours**	**72 hours**	**96 hours**
Control	--	-	-	-
1.7	0.67	-6.0	-7.4	-15
3.1	4.7	-13	-17	-5.3
6.8	1.9	14	16*	3.3
14	29	48	61*	31*
31	39	69	86*	81*

** significantly different from control ($p < 0.05$)*

Table B.9.2.6-6: Effect of CGA 71019 on algal biomass

Mean measured concentration (mg/L)	Mean biomass (% inhibition relative to control)			
	24 hours	48 hours	72 hours	96 hours
Control	-	-	-	-
1.7	0.74	-4.8	-6.9	-10
3.1	5.2	-9.6	-16	-12
6.8	2.1	12	15*	10*
14	32	45	57*	47*
31	43	65	81*	82*

**significantly different from control ($p < 0.05$)*

Table B.9.2.6-7: Effect of CGA 71019 on algal growth rate

Mean measured concentration (mg/L)	Growth rate (% inhibition relative to control)			
	24 hours	48 hours	72 hours	96 hours
Control	-	-	-	-
1.7	0.40	-1.3	-1.1	-2.0
3.1	2.1	-2.7	-2.6	-0.76
6.8	0.85	3.5	2.9*	0.51
14	14	15	15*	5.5*
31	21	26	32*	25*

** significantly different from control ($p < 0.05$)*

Table B.9.2.6-8: EC_{50} values over the 96-hour exposure period

Time	Cell Density		Biomass		Growth Rate	
	EC_{50} (mg/L)	95% CI (mg/L)	EC_{50} (mg/L)	95% CI (mg/L)	EC_{50} (mg/L)	95% CI (mg/L)
24 Hours	>31	n.d.	>31	n.d.	>31	n.d.
48 Hours	16	13-19	18	15-21	>31	n.d.
72 Hours	12	9.9-14	13	11-15	>31	n.d.
96 Hours	18	16-19	14	13-16	>31	n.d.

n.d. 95% confidence interval could not be determined.

Based on mean measured concentrations and cell density, the 72-hour EC_{50} of CGA 71019 for *Pseudokirchneriella subcapitata* was 13 mg/L. The corresponding value based on growth rate was >31 mg/L.

RMS comments:

The study was conducted in accordance with the referred guidelines and is considered to be valid for the risk assessment.

Reference:	**Swarbrick, R.H. (2001b). CGA 205375: Toxicity to the green alga *Selenastrum capricornutum*. Brixham Environmental Laboratory, UK. Unpublished report no. BL7203/B. (Syngenta File No 205375/0015)**
Guideline:	US EPA OPPTS 850.5400.
GLP:	Yes.

Material and methods:

Test substance:	CGA 205375, batch number MLA-421/2, purity 99%.
Species:	Green algae (*Selenastrum capricornutum/Pseudokirchneriella subcapitata*).
Treatments:	Nominal concentrations were 0.1, 0.18, 0.23, 0.56, 1.0, 1.8, 3.2, 5.6 mg/L of CGA 205375. Cultures were treated with aliquots of a stock solution containing CGA 205375 prepared in water. The test incorporated three replicate cultures of each exposure concentration dose and six replicates of an untreated control.

Initial cell density:	1.03 x 10^4 cells/mL.
Duration:	96 hours, static test.
Test conditions:	Temperature 24±1°C, pH 7.4 – 9.3. Continuous light.
Observations:	Cell density was assessed daily using a cell counter. At 0 and 96 hours, pH was recorded and water samples were collected for analysis of test substance concentration by HPLC.
Data analysis:	One-way analysis of variance, Dunnet's test.

Results:

Mean measured CGA 205375 concentrations corresponded to 82-150% of nominal concentrations. Mean cell densities and inhibition values are presented in the table below.

Table B.9.2.6-9: Effect of CGA 205375 on algal cell density.

Nominal concentration (mg/L)	Mean measured concentration (mg/L)	Mean cell density (cells/mL*10000)			
		24 hour	48 hour	72 hour	96 hour
Control	<0.0064	3.4	20.8	115	360
0.1	0.15	3.83	20.8	111	334
0.18	0.18	3.77	22.9	121	369
0.32	0.32	3.63	18.3	94.4	318
0.56	0.60	3.52	17.9	89.9	298
1.0	0.99	3.23	15.8	72.1	245
1.8	1.8	2.83	8.11	23.7	77.4
3.2	3.3	1.82	3.72	7.11	12.3
5.6	4.6	1.72	2.55	3.66	6.31
E_bC_{50}	n.a.	2.78	1.70	1.24	1.23
95% confidence interval (p≤0.05)	n.a.	2.69-2.88	1.66-1.73	1.19-1.30	1.18-1.28
E_rC_{50}	n.a.	3.67	2.96	2.82	3.09
95% confidence interval (p≤0.05)	n.a.	2.91-4.42	2.64-3.28	2.77-2.87	3.01-3.17

n.a. not applicable

Based on mean measured concentrations, 96-hour E_bC_{50} for CGA 205375 in *Pseudokirchneriella subcapitata* was estimated to be 1.23 mg/L. The corresponding value based on growth rate was 3.09 mg/L.

RMS comments:

The study was conducted in accordance with the referred guidelines. Since the exponential growth phase tended to cease in the control after 96 hours, it seems more appropriate to use the EC_{50} values from 72 hours. This is also in line with the recommended study duration in the OECD guidelines 201. In conclusion, the 72-h EC_{50} values of 1.2 (1.2-1.3) mg/L for biomass and 3.1(3.0-3.2) mg/L for growth rate will be used in the risk assessment.

FORMULATED PRODUCTS

DIVIDEND 030FS

Reference:	**Gries, T. (1999c). Toxicity test of CGA 169374 FS(30) (A-9142 G) to the freshwater alga (*Pseudokirchneriella subcapitata*). Springborn Laboratories, Horn, Switzerland. Unpublished report no.1047.062.430. Study dates 15-18 June 1999 (Syngenta File No. CGA 169374/1906)**

Guideline:	OECD 201 (1984).
GLP:	Yes.

Material and methods:

Test substance:	A-9142 G containing 30.6 g/L CGA 169374 (density 1049 kg/m^3). Batch number P.902001.
Species:	Green alga (*Pseudokirchneriella subcapitata*)
Treatments:	Nominal concentrations were 6.25, 12.5, 25, 50 and 100 mg formulation/L. The test incorporated three replicate cultures for each dose and an untreated control.
Initial cell density:	10000 cells/mL.
Duration:	72 hours, static test.
Test conditions:	Temperature 23.5-25.4°C, pH 7.74-9.04. Conductivity 147-178 µS/cm. Continuous light, 6000-7200 lux.
Observations:	Cell density was assessed daily using a cell counter. Temperature was monitored continuously while pH and conductivity were recorded at test initiation and test termination. Test substance concentrations were determined by chemical analysis of test solutions sampled immediately before exposure was initiated.
Data analysis:	Shapiro-Wilk's test, Bartlett's test, Dunnett's test.

Results:

Mean initial test substance concentrations corresponded to 80.3-99.2% of nominal concentrations. Cell density data are presented in the table below.

Table B.9.2.6-10: Effect of A-9142 G on algal cell density.

Nominal concentration (mg/L)	Mean cell density (cells/mL*10000)			Mean inhibition (%)
	24 hour	48 hour	72 hour	0-72 hour
0	4.83	24.83	90.33	0
6.25	5.25	24.58	93.17	-3.14
12.5	5.08	21.00	91.08	-.083
25	5.00	21.58	91.00	-0.74
50	3.25	14.33	38.50	57.38
100	3.33	7.17	11.42	87.36

Based on nominal concentrations, the 72-hour E_bC_{50} and E_rC_{50} values for A-9142 G to *Pseudokirchneriella subcapitata* were 62 (42-87) and 112 (92-141) mg formulation/L, respectively. These values are equivalent to 1.81 and 3.27 mg as/L, respectively.

RMS comments:

Although the highest test concentration was not sufficient to reach >50% effect on the specific growth rate, the study was generally conducted in accordance with the referred guidelines. Since the growth rate EC_{50} was extrapolated, the value will be set to >100 mg/L (highest tested concentration).

SCORE 250EC

Reference:	**Peters (1992). Toxicity of CGD 96430F to *Scenedesmus subspicatus* CHODAT (96 hours). Okolimna, Burgwedel, Germany. Unpublished report number 04/92317. Experimental phase 4 June – 4 October 1992. Syngenta File N° CGA169374/0630**
Guideline:	OECD 201 (1984).
GLP:	Yes.

Material and methods:

Test substance:	CGD 96430 F (equivalent to A-7402 A), containing 250 g CGA 169374/L**.** Batch number P.111003.
Species:	Green algae *Scenedesmus subspicatus*.
Treatments:	Nominal test concentrations were 3.29, 4.94, 7.41, 11.11, 16.67 and 25 mg formulation/L. The test incorporated three replicate cultures for each dose and an untreated control.
Initial cell density:	10000 cells/mL
Duration:	96 hours, static test.
Test conditions:	Temperature 22.5 ± 1°C, pH 7.5 – 8.3. Continuous light 8000 Lux.
Observations:	Cell density was assessed daily using a fluorimeter and pH was recorded at 0, 72 and 96 hours.
Data analysis:	Graphical determination and probit analysis.

Results:

Mean cell densities and inhibition values are presented in the table below.

Table B.9.2.6-11: Effect of A-7402 A on algal cell density

Nominal concentration (mg formulation/L)	**Fluorescence**				**Mean inhibition (%)**
	24 hour	48 hour	72 hour	96 hour	0-96 hour
0	0.06	0.24	0.47	0.72	-
3.29	0.06	0.20	0.38	0.58	12.77
4.94	0.07	0.22	0.37	0.58	16.16
7.41	0.06	0.18	0.26	0.38	49.20
11.11	0.05	0.06	0.04	0.05	91.49
16.67	0.04	0.02	0.01	0.02	102.80
25.00	0.03	0.01	0.00	0.00	105.82

Based on nominal concentrations, the 96-hour E_bC_{50} and E_rC_{50} for CDG 96430F in *Scenedesmus subspicatus* were estimated to be 6.6 and 8.6 mg formulation/L. These values are equivalent to 1.6 and 2.15 mg as/L, respectively.

RMS comments:

No analytical measurements of the test concentrations were reported in the study. Hence, the results from this study should be treated with caution. However, the results indicate that the formulation is not more toxic than the active ingredient alone. No further data is considered necessary, since the risk assessment will be driven by the much lower EC_{50} value from the active ingredient study by Grade (1993b).

Reference:	**Grade, R. (1994b). Report on the growth inhibition test of CGA 169374 EC 250 (A-7402 H) to green algae (*Scenedesmus subspicatus*). Ciba Geigy Ltd., Basel Switzerland. Unpublished report number 943541. Experimental phase 19 April – 18 May 1994. Syngenta File N° CGA169374/0948**
Guideline:	OECD 201 (1984).
GLP:	Yes.

Material and methods:

Test substance:	A-7402 H containing 250 g CGA 169374/L**.** Batch number P.308002; Density 1.0129 kg/L.
Species:	Green algae (*Scenedesmus subspicatus*).
Treatments:	Nominal concentrations were 0.044, 0.096, 0.21, 0.46, 1.0, and 2.2 mg/L. The test incorporated three replicate cultures for each dose and six replicate cultures of an untreated control.
Initial cell density:	10400 cells/mL.
Duration:	72 hours, static test.
Test conditions:	Temperature 23 ± 1°C, pH 7.7 – 8.1. Continuous light 8000 lux.
Observations:	Cell density was assessed daily using a cell counter. pH was recorded at 0 and 72 hours and water samples were collected at test initiation and test termination for analysis of test substance concentration by gas chromatography.
Data analysis:	Maximum likelihood model, logit model.

Results:

Initial mean measured difenoconazole concentrations corresponded to 89-100% of nominal concentrations. Mean cell densities and inhibition values are presented in the table below.

Table B.9.2.6-12: Effect of A-7402 H on algal cell density

Mean measured concentration (mg formulation/L)	Mean cell density (cells/mL*10000)			Mean inhibition (%)
	24 hour	48 hour	72 hour	0-72 hour
0	5.7	26.5	126.3	0
0.029	5.7	23.8	106.3	13.7
0.063	4.4	23.0	125.5	5.6
0.14	4.3	13.6	70.2	45.7
0.34	2.9	3.5	8.5	91.4
0.70	2.8	2.3	4.3	95.0
1.90	1.9	1.8	3.0	97.2

Based on measured concentrations, the 72-hour E_bC_{50} for A-7402 H in *Scenedesmus subspicatus* was estimated to be 0.15 mg/L (confidence interval, p≤0.05, 0.13-0.19 mg/L), equivalent to 0.037 mg as/L.

RMS comments:

This study was performed with a formulation that is not considered to correspond to the proposed representative formulation A-7402 T. The results will therefore not be used in the further assessment.

Reference:	**Volz, E. (2004) Difenoconazole 250 EC formulation (A7402T): A 72-hour algal growth inhibition test with *Scenedesmus subspicatus*. RCC Ltd, Environmental Chemistry & Pharmanalytics, CH-4452 Itingen / Switzerland. Unpublished**

	Report No. 853037. Study dates: April 30, 2004 – May 12, 2004 (Syngenta file No. CGA169374/2553)
Guideline:	OECD 201 (1984).
GLP:	Yes (certified laboratory).

Material and methods:

Test substance:	The formulation tested was difenoconazole 250 EC formulation (A-7402 T); batch no. SEZ3DP001; content of active ingredient difenoconazole (CGA169374): 252 g/L.
Species:	Green algae *Scenedesmus subspicatus*
Treatments:	Exponentially growing algal cultures were exposed to seven nominal test item concentrations of 0.10, 0.22, 0.46, 1.0, 2.2, 4.6 and 10 mg/L A7402T.
Initial cell density:	Initial cell density was 10000 cells per mL. The test design included three replicates per test concentration and six replicates of the control.
Duration:	72 hours, static test.
Test conditions:	The total hardness of the test water was calculated to be 24 mg $CaCO_3$/L. During the study the water temperature was in the range from 22 to 23 °C and the light intensity was about 6300 Lux (mean value), range: 5930 to 6610 Lux. The pH value was 7.9 at the start of the test and ranged from 8.1 and 9.1 at the end of the test.
Observations:	The algal cell densities were determined after 24, 48, and 72 hours test duration. The temperature was measured at 24-hour intervals. The pH was measured at the start and the end of the experiment. Analytical determinations of the toxicant concentrations were made at the start and end of the experiment.
Data analysis:	Dunnett's test.

Results:

The measured test item concentrations (based on the analytical measurement of difenoconazole) at the start of the test ranged from 80 to 96% of the nominal values. During the test period of 72 hours a decrease of the concentrations of difenoconazole in the test media was observed, and at the end of the study, the measured concentrations were below 80% in four of the seven test concentrations. The mean measured test concentrations (average over all measurements per test concentration) varied in the range of 82 to 91% of the nominal values. All reported biological results are based on the nominal concentrations. The inhibition in growth of the algae (biomass and growth rate) after the test duration of 72 hours has been presented in the table below.

Table 9.2.6-13: Influence of A-7402 T on the growth of *Scenedesmus subspicatus*

Concentration (mg A-7402 T /L)	**Growth inhibition (%) compared to control** 72h	
Nominal	Biomass	Growth rate
Control	—	—
0.10	4.8	0.7
0.22	4.8	1.0
0.46	24.9	8.5
1.0	26.5	9.8
2.2	44.0	17.6
4.6	82.7	46.9
10	97.1	84.5
(all values in mg A-7402 T /L)		
EC_{50}	1.7	4.4
95% C.I.	1.0–2.9	2.8–8.4
NOEC	0.22	0.22
LOEC	0.46	0.46

In conclusion, the 72-hour EC_{50} (growth rate) of A-7402 T to *Scenedesmus subspicatus* was 4.4 mg/L.

RMS comments:

Due to the decreased test concentrations at the end of the study, the results should be based on mean measured concentrations. Recalculations based on mean measured concentrations would give a 72-hour EC_{50} value of 1.41 (95%CL 0.88 – 2.38) mg formulation/L, corresponding to 0.29 (0.22 – 0.60) mg as/L for biomass, and 3.82 (95%CL 2.49 - 7.01) mg formulation/L, corresponding to 0.96 (0.62 – 1.75) mg as/L for growth rate. These values will be used in the risk assessment.

The notifier stated that for formulation studies it is more appropriate to calculate the results based on nominal concentrations. However, the formulation studies in this case is used to assess the risk from the active ingredient, and for precautionary reasons it is assumed that the total toxicity derives from difenoconazole, the RMS maintains that the results should be based on mean measured concentrations. This is also in line with current practice.

B.9.2.7 Effects on higher aquatic plants

Reference:	**Drottar, K.R. (1986). Acute toxicity of CGA 169374 to duckweed (*Lemna gibba* G3). ERT, Fort Collins, Colorado, USA. Unpublished report no. D842-100. (Syngenta File No 169374/0025)**
Guideline:	US EPA FIFRA 122-2.
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number FL 851406, purity 96.1%.
Species:	Duckweed (*Lemna gibba* G3)
Treatments:	Nominal concentrations 1.25, 2.5, 5, 10, 20 and 40 mg/L. Aliquots of a difenoconazole stock solution prepared in methanol were added to *Lemna* cultures in nutrient medium.
Initial frond number:	5 plants per culture, each with 3 fronds. The test incorporated three replicate cultures

	for each dose, six replicate cultures of a solvent control prepared with methanol (0.2 mL/L) and six replicates of an untreated control.
Duration:	14 days, static renewal system.
Test conditions:	Temperature 25°C, pH not reported. Continuous light, 5000 lux. Test solutions were renewed on days 4, 7 and 11.
Observations:	Frond number and dry weight biomass was assessed after 14 days.
Data analysis:	Student's t-test, probit analysis, analysis of variances.

Results:

The effects of difenoconazole on frond number and dry weight are presented in the table below.

Table B.9.2.7-1: Effect of difenoconazole on frond production in *Lemna gibba.*

Nominal concentration (mg/L)	14 day frond number		14 day dry weight (g)	
	Total	% reduction	Total	% reduction
Solvent control	1301	-	0.5178	-
1.25	1611	-24	0.5375	-3.8
2.5	1491	-15	0.4719	8.9
5	1386	-6	0.3121	39.7
10	1113	14.5	0.2797	46.0
20	405	68.9	0.1230	76.3
40	235	81.9	0.0746	85.6

Based on nominal concentrations, the 14-day EC_{50} for frond number and dry weight were 18.5 (95% confidence interval ($p \leq 0.05$) of 18.0 to 19.0 mg/L) and 9.9 mg/L (95% confidence interval ($p \leq 0.05$) of 9.7 to 10.1 mg/L), respectively.

RMS comments:

Since no analytical measurements were made to verify the test concentrations, the results should be treated with caution. The results will not be used in the risk assessment. However, the results indicate that *Lemna* is less sensitive to difenoconazole that the unicellular green algae tested. Further, since difenoconazole is not a herbicide or growth regulator, a test on higher aquatic plants is not formally required.

B.9.2.8 Summary of toxicity studies on aquatic organisms

Descriptions of aquatic toxicity studies conducted with difenoconazole and the metabolites CGA 205375 and CGA 71019 are provided in **Document M-II, Section 6**.

Toxicity studies with fish, daphnia and algae were conducted with two precursor formulations (A-7402 H and A-7402 A) that are similar to A-7042 T (SCORE 250EC), and a *Chironomus* study was conducted with A-7042 G. Descriptions of these studies are provided **Document M-III for SCORE 250EC, Section 6**.

The acute toxicity studies with fish, *Daphnia* and algae were also conducted with A-9142 G (DIVIDEND 030FS). Descriptions of these studies are provided **Document M-III for DIVIDEND 030FS, Section 6.**

A summary of all endpoints is provided in the table below.

Table B.9.2.8-1: Toxicity of difenoconazole, its major metabolites in water/sediment systems and the representative formulations DIVIDEND 030FS and SCORE 250EC to aquatic organisms.

Study type Test substance	Species	Endpoint	Value (mg as/L)	Reference
FISH ACUTE TOXICITY				
Difenoconazole	Rainbow trout	96 h LC_{50}	1.1(0.98-1.1)	Surprenant (1990a)
	Bluegill sunfish	96 h LC_{50}	1.3 (1.0-1.8)	Bowman (1988)
	Sheepshead minnow	96 h LC_{50}	1.1(0.86-1.5)	Machado (1993)
CGA 71019	Rainbow trout	96-h LC_{50}	498 (378-657)	Rufli (1983)
CGA 205375	Rainbow trout	96-h LC_{50}	0.74 (0.58-0.95)	Swarbrick (2001a)
DIVIDEND 030FS	Rainbow trout	96-h LC_{50}	0.70 (0.43-1.2)	Gries (1999a)
SCORE 250EC	Rainbow trout	96-h LC_{50}	**0.65** (0.56-1.1)	Voigt (1990a)
SCORE 250EC	Rainbow trout	96-h LC_{50}	0.38 – 0.92	Volz (2004a)
FISH PROLONGED TOXICITY				
Difenoconazole	Rainbow trout	21-day NOEC	0.023	Grade (1993a)
CGA 71019	Rainbow trout	28-d NOEC	3.2	Dorgerloh and Sommer (2002)
CGA 205375	No data available	not needed		
DIVIDEND 030FS	No data available	not needed		
SCORE 250EC	Rainbow trout	21-day NOEC	0.15	Voigt (1991)
FISH EARLY LIFE STAGE TOXICITY				
Difenoconazole	Fathead minnow (ELS)	NOEC	**0.0076**	Surprenant (1987b)
	Fathead minnow (ELS)	NOEC	0.0087	Surprenant (1990b)
BIOCONCENTRATION IN FISH				
Difenoconazole (20 ug/L)	Bluegill sunfish	BCF (whole fish)	320±32 (no unit)	Forbis (1987)
Difenoconazole (1 ug/L)	Bluegill sunfish	BCF (whole fish)	330 (no unit)	Fackler (1992)
INVERTEBRATES ACUTE				
Difenoconazole	*Daphnia magna*	48 h LC_{50}	0.77 (0.59-0.95)	Forbis (1988a)
	*Mysidopsis bahia**	96 h LC_{50}	**0.15** (0.11-0.22)	Suprenant (1990b)
	*Crassostrea virginica**	96 h LC_{50}	>0.3	Surprenant (1990c)
CGA 71019	*Daphnia magna*	48-h EC_{50}	>100	Bell (1995)
CGA 205375	*Daphnia magna*	48 h LC_{50}	1.4 (1.2-1.7)	Swarbrick (2001b)
DIVIDEND 030FS	*Daphnia magna*	48 h LC_{50}	0.43 (0.3-0.6)	Gries (1999b)
SCORE 250EC	*Daphnia magna*	48 h LC_{50}	0.62 – 1.38	Volz (2004b)
INVERTEBRATES CHRONIC				
Difenoconazole	*Daphnia magna*	21-day NOEC	**0.0056**	Forbis (1988b)
	Chironomus riparius	28-d NOEC	0.015 (*via* water)	Van der Kolk (1999)
	Chironomus riparius	28-d NOEC	50 mg as/kg sediment	Van der Kolk (1999)
CGA 71019	No data available	not needed		
CGA 205375	*Chironomus riparius*	28-d NOEC	0.4 (via water)	Grade (2001)
	Chironomus riparius	28-d NOEC	10 mg/kg (via sediment)	Grade (2001)
SCORE 250EC	*Chironomus riparius*	28-day NOEC	0.075 (*via* water)	Neumann (1997)
DIVIDEND 030FS	No data available	not needed		
ALGAE				
Difenoconazole	*Scenedesmus subspicatus*	72-h E_bC_{50}/E_rC_{50}	1.2/3.8 (3.3-4.5)	Rufli (1989)
	Scenedesmus subspicatus	72-h E_bC_{50}/E_rC_{50}	**0.032** (0.026-0-039)	Grade (1993b)
CGA 71019	*Pseudokirchneriella subcapitata*	72-h E_bC_{50}/E_rC_{50}	13 (11-15)	Palmer *et al* (2001)
CGA 205375	*Pseudokirchneriella subcapitata*	72-h E_bC_{50}/ E_rC_{50}	1.2(1.2-1.3)/ 3.1(3.0-3.2)	Swarbrick (2001b)
DIVIDEND 030FS	*Scenedesmus subspicatus*	72-h E_bC_{50}/ E_rC_{50}	1.8(1.3-2.6)/ >3.0(2.8->3.0)	Gries (1999c)
SCORE 250EC	*Scenedesmus subspicatus*	96-h E_bC_{50}/E_rC_{50}	1.6/2.5	Peters (1992)

SCORE 250EC	*Scenedesmus subspicatus*	72-h E_bC_{50}/E_rC_{50}	0.29 (0.22 – 0.60)/ 0.96 (0.62 – 1.75)	Volz (2004c)
AQUATIC PLANTS				
Difenoconazole	No reliable data	not required		

[a] *LC_{50} = median lethal concentration (50% mortality); EC_{50} = median effect concentration (50% effects); E_bC_{50} = effective concentration for 50% biomass reduction; NOEC = no observed effect concentration*
**marine species*

For the metabolite CGA 71019, long term data was available for fish, although the acute test on invertebrates did not include concentrations high enough to conclude which species (fish or invertebrates) were the most sensitive. However, since the risk assessment resulted in very high TER values for the metabolite (acute as well as long term for fish), there is a sufficient safety margin and no further data is considered to be necessary.

Regarding the metabolite CGA 205375, fish was slightly more sensitive that invertebrates in acute studies. However, since the major part of this compound has been concluded to be situated in the sediment (see Annex B.8), the RMS considers that it is justified to focus the long term assessment on the sediment dwelling invertebrate *Chironomus*.

No long term data was available for DIVIDEND 030FS, and this is not needed since this is a seed treatment formulation that will not directly reach surface waters, and continued or repeated exposure will not occur.

In conclusion, the available studies are considered to fulfil the data requirements of Annex II and III of 91/414 and are sufficient for the risk assessment for aquatic organisms.

B.9.2.9 Risk assessment for aquatic organisms

B.9.2.9.1 SEED TREATMENT WITH DIVIDEND 030FS

A risk assessment for aquatic organisms at the use of difenoconazole for seed treatment of wheat with the formulation DIVIDEND 030FS was provided by the Notifier in Document M-III, section 6.10. The PEC_{sw} values were slightly corrected based on the RMS assessment in Annex B.8, and the calculated TER values below are based on the RMS's values.

Table B.9.2.9-1: Toxicity/exposure ratios for the most sensitive aquatic organisms based on FOCUS Step 1. Seed treatment of wheat, 60 mg as/kg seed.

Test substance	Organism	Toxicity end point (mg as/L)	Time scale	PEC_i (mg/L)	TER	Annex VI Trigger[1]
SCORE 250EC	Rainbow trout	0.65	Acute	0.00069	942	100
difenoconazole	Fathead minnow (ELS)	0.0076	Chronic	0.00069	11	10
difenoconazole	*Mysidopsis bahia*	0.15	Acute	0.00069	217	100
difenoconazole	*Daphnia magna*	0.0056	Chronic	0.00069	**8.1**	10
difenoconazole	*Scenedesmus subspicatus*	0.032	Chronic	0.00069	46	10
difenoconazole	*Chironomus riparius*	0.015 (water)	Chronic	0.00069	22	10
CGA 71019	Rainbow trout	498	Acute	0.00015	3320000	100

Test substance	Organism	Toxicity end point (mg as/L)	Time scale	PEC_i (mg/L)	TER	Annex VI Trigger[1]
CGA 71019	Rainbow trout	3.2	Chronic	0.00015	21333	10
CGA 71019	*Daphnia magna*	>100	Acute	0.00015	666667	100
CGA 71019	Aquatic invertebrates	-	Chronic	0.00015	-	10
CGA 71019	*Pseudokirchneriella subcapitata*	13	Chronic	0.00015	86667	10
CGA 205375	Rainbow trout	0.74	Acute	0.000068	10882	100
CGA 205375	Fish	-	Chronic	0.000068	-	10
CGA 205375	*Daphnia magna*	1.4	Acute	0.000068	20588	100
CGA 205375	*Chironomus riparius*	0.4 (water)	Chronic	0.000068	5882	10
CGA 205375	*Pseudokirchneriella subcapitata*	1.2	Chronic	0.000068	17647	10

From the TER values calculated using initial PEC from FOCUS Step 1 (see Annex B.8), it is concluded that no refinement is needed for the short term and long term assessment for fish and sediment dwelling species or short term assessment for aquatic invertebrates, or for algae regarding the active ingredient, or for any trophic level regarding the major metabolites. However, further refinement is needed for the long term risk from difenoconazole to aquatic invertebrates.

Table B.9.2.9-2: Toxicity/exposure ratios for the most sensitive aquatic organisms based on FOCUS Step 2. Seed treatment of wheat, 60 mg as/kg seed, autumn, Northern Europe (worst case, covers also spring application in Northern and Southern EU).

Test substance	N/S	Organism	Toxicity end point (mg as/L)	Time scale	PEC_i (mg/L)	TER	Annex VI Trigger[1]
difenoconazole	N	*Daphnia magna*	0.0056	Chronic	0.00034	16	10

In conclusion, based on FOCUS Step 2 PECsw, all TER values are above the trigger values in Annex VI of 91/414, and no further refinement is needed.

B.9.2.9.2 SPRAY APPLICATION WITH SCORE 250EC

A risk assessment for aquatic organisms at the use of difenoconazole for spray application of carrots and pome fruit with the formulation SCORE 250EC was provided by the Notifier in Document M-III, section 6.10. The PEC_{sw} values were slightly corrected based on the RMS assessment in Annex B.8, and the calculated TER values below are based on the RMS's values.

Table B.9.2.9-3: Toxicity/exposure ratios for the most sensitive aquatic organisms based on FOCUS Step 1. Carrots, 3 x 125 g as/ha, 14 days interval between treatments.

Test substance	Organism	Toxicity end point (mg as/L)	Time scale	PEC_i (mg/L)	TER	Annex VI Trigger[1]
SCORE 250EC	Rainbow trout	0.65	Acute	0.024	**27**	100
difenoconazole	Fathead minnow (ELS)	0.0076	Chronic	0.024	**0.32**	10
difenoconazole	*Mysidopsis bahia*	0.15	Acute	0.024	**6.3**	100
difenoconazole	*Daphnia magna*	0.0056	Chronic	0.024	**0.23**	10
difenoconazole	*Scenedesmus subspicatus*	0.032	Chronic	0.024	**1.3**	10

Test substance	Organism	Toxicity end point (mg as/L)	Time scale	PEC_i (mg/L)	TER	Annex VI Trigger[1]
difenoconazole	*Chironomus riparius*	0.015	Chronic	0.024	**0.63**	10
CGA 71019	Rainbow trout	498	Acute	0.0044	113182	100
CGA 71019	Rainbow trout	3.2	Chronic	0.0044	727	10
CGA 71019	*Daphnia magna*	>100	Acute	0.0044	22727	100
CGA 71019	Aquatic invertebrates	-	Chronic	0.0044	-	10
CGA 71019	*Pseudokirchneriella subcapitata*	13	Chronic	0.0044	2955	10
CGA 205375	Rainbow trout	0.74	Acute	0.0024	308	100
CGA 205375	Fish	-	Chronic	0.0024	-	10
CGA 205375	*Daphnia magna*	1.4	Acute	0.0024	583	100
CGA 205375	*Chironomus riparius*	0.4	Chronic	0.0024	167	10
CGA 205375	*Pseudokirchneriella subcapitata*	1.2	Chronic	0.0024	500	10
CGA 205375	Sediment organisms	0.4	Chronic	0.0024	167	10

Table B.9.2.9-4: Toxicity/exposure ratios for the most sensitive aquatic organisms based on FOCUS Step 1. Pome fruit Southern EU, 4 x 75 g as/ha, 7 days interval between treatments (covers also pome fruit in Northern EU, 4 x 56.25 g as/ha, 7 days interval between treatments).

Test substance	Organism	Toxicity end point (mg as/L)	Time scale	PEC_i (mg/L)	TER	Annex VI Trigger[1]
SCORE 250EC	Rainbow trout	0.65	Acute	0.032	**20**	100
difenoconazole	Fathead minnow (ELS)	0.0076	Chronic	0.032	**0.2**	10
difenoconazole	*Mysidopsis bahia*	0.15	Acute	0.032	**4.7**	100
difenoconazole	*Daphnia magna*	0.0056	Chronic	0.032	**0.2**	10
difenoconazole	*Scenedesmus subspicatus*	0.032	Chronic	0.032	**1.0**	10
difenoconazole	*Chironomus riparius*	0.015	Chronic	0.032	**0.5**	10
CGA 71019	Rainbow trout	498	Acute	0.0038	131053	100
CGA 71019	Rainbow trout	3.2	Chronic	0.0038	842	10
CGA 71019	*Daphnia magna*	>100	Acute	0.0038	26316	100
CGA 71019	Aquatic invertebrates	-	Chronic	0.0038	-	10
CGA 71019	*Pseudokirchneriella subcapitata*	13	Chronic	0.0038	3421	10
CGA 205375	Rainbow trout	0.74	Acute	0.0032	231	100
CGA 205375	Fish	-	Chronic	0.0032	-	10
CGA 205375	*Daphnia magna*	1.4	Acute	0.0032	438	100
CGA 205375	*Chironomus riparius*	0.4	Chronic	0.0032	125	10
CGA 205375	*Pseudokirchneriella subcapitata*	1.2	Chronic	0.0032	375	10
CGA 205375	Sediment organisms	0.4	Chronic	0.0032	125	10

Based on the FOCUS Step 1 PECsw values, the TER triggers were higher than the trigger values in Annex VI of 91/414 for the major metabolites. However, for difenoconazole refinement was needed for all trophic levels at the representative use in carrots and pome fruit.

Table B.9.2.9-5: Toxicity/exposure ratios for the most sensitive aquatic organisms based on FOCUS Step 2. Carrots, 3 x 125 g as/ha, 14 days interval between treatments (covers also pome fruit in Northern EU, 4 x 56.25 g as/ha, 7 days interval between treatments).

Test substance	N/S	Organism	Toxicity end point (mg as/L)	Time scale	PEC_i (mg/L)	TER	Annex VI Trigger[1]
SCORE 250EC	S	Rainbow trout	0.65	Acute	0.0027	241	100
difenoconazole	S	Fathead minnow (ELS)	0.0076	Chronic	0.0027	**2.8**	10
difenoconazole	S	*Mysidopsis bahia*	0.15	Acute	0.0027	**56**	100

Test substance	N/S	Organism	Toxicity end point (mg as/L)	Time scale	PEC_i (mg/L)	TER	Annex VI Trigger[1]
difenoconazole	S	*Daphnia magna*	0.0056	Chronic	0.0027	**2.1**	10
difenoconazole	S	*Scenedesmus subspicatus*	0.032	Chronic	0.0027	12	10
difenoconazole	S	*Chironomus riparius*	0.015	Chronic	0.0027	**5.6**	10

Table B.9.2.9-6: Toxicity/exposure ratios for the most sensitive aquatic organisms based on FOCUS Step 2. Pome fruit Southern EU, 4 x 75 g as/ha, 7 days interval between treatments.

Test substance	N/S	Organism	Toxicity end point (mg as/L)	Time scale	PEC_i (mg/L)	TER	Annex VI Trigger[1]
SCORE 250EC	S	Rainbow trout	0.65	Acute	0.0042	155	100
difenoconazole	S	Fathead minnow (ELS)	0.0076	Chronic	0.0042	**1.8**	10
difenoconazole	S	*Mysidopsis bahia*	0.15	Acute	0.0042	**36**	100
difenoconazole	S	*Daphnia magna*	0.0056	Chronic	0.0042	**1.3**	10
difenoconazole	S	*Scenedesmus subspicatus*	0.032	Chronic	0.0042	**7.6**	10
difenoconazole	S	*Chironomus riparius*	0.015	Chronic	0.0042	**3.6**	10

Based on the FOCUS Step 2 PECsw values for the representative use in carrots, the TER values were higher than the trigger values in Annex VI of 91/414 for short term effects on fish and for algae. Refinement was needed for long term risk to fish, for short and long term risk to aquatic invertebrates and for sediment dwelling organisms. For pome fruit, the TER values were higher than the trigger values in Annex VI of 91/414 for short term effects on fish. Refinement was needed for long term risk to fish, for short and long term risk to aquatic invertebrates and for sediment dwelling organisms and for algae.

The most sensitive species tested was *Daphnia magna*, and it is considered that the refined assessment based on this NOEC value will cover also the other trophic levels which failed the lower tier assessments.

Table B.9.2.9-7: Refined aquatic risk assessment using Step 3 FOCUS modelling. Carrots, 3 x 125 g as/ha, 14 days interval between treatments (covers also pome fruit in Northern EU, 4 x 56.25 g as/ha, 7 days interval between treatments).

Test substance	Scenario[1]	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw global max. (mg/L)	TER	Annex VI trigger
difenoconazole	D3	ditch	*Daphnia magna*	long term	0.0056	0.000573	**9.8**	10
difenoconazole	D6	ditch	*Daphnia magna*	long term	0.0056	0.000570	**9.8**	10
difenoconazole	R1	pond	*Daphnia magna*	long term	0.0056	0.000082	68	10
difenoconazole	R1	stream	*Daphnia magna*	long term	0.0056	0.000376	15	10
difenoconazole	R2	stream	*Daphnia magna*	long term	0.0056	0.000504	11	10
difenoconazole	R3	stream	*Daphnia magna*	long term	0.0056	0.000530	11	10
difenoconazole	R4	stream	*Daphnia magna*	long term	0.0056	0.000713	**7.9**	10

[1] *drainage (D1-D6) and run-off (R1-R4)*

Table B.9.2.9-8: Refined aquatic risk assessment using Step 3 FOCUS modelling. Pome fruit Southern EU, 4 x 75 g as/ha, 7 days interval between treatments

Test substance	Scenario[1]	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw global max. (mg/L)	TER	Annex VI trigger
difenoconazole	D3	ditch	*Daphnia magna*	long term	0.0056	0.00179	**3.1**	10
difenoconazole	D4	pond	*Daphnia magna*	long term	0.0056	0.000241	23	10

Test substance	Scenario[1]	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw global max. (mg/L)	TER	Annex VI trigger
difenoconazole	D4	stream	*Daphnia magna*	long term	0.0056	0.00168	**3.3**	10
difenoconazole	D5	pond	*Daphnia magna*	long term	0.0056	0.000240	23	10
difenoconazole	D5	stream	*Daphnia magna*	long term	0.0056	0.00181	**3.1**	10
difenoconazole	R1	pond	*Daphnia magna*	long term	0.0056	0.000227	25	10
difenoconazole	R1	stream	*Daphnia magna*	long term	0.0056	0.00137	**4.1**	10
difenoconazole	R2	stream	*Daphnia magna*	long term	0.0056	0.00182	**3.1**	10
difenoconazole	R3	stream	*Daphnia magna*	long term	0.0056	0.00194	**2.9**	10
difenoconazole	R4	stream	*Daphnia magna*	long term	0.0056	0.00138	**4.1**	10

[1] *drainage (D1-D6) and run-off (R1-R4)*

Chronic TERs for the most sensitive species based on the maximum PEC from FOCUS Step 3 still fall below relevant triggers for pome fruit (all scenarios) and carrots (3 out of 6 scenarios), indicating the need for further refinement.

The notifier claimed that the use of maximum PEC_{sw} values for assessing chronic risk of difenoconazole is very conservative, since in natural aquatic systems difenoconazole will rapidly dissipate from the water column (DT_{50} up to 2 days in the water phase in water-sediment studies). The test designs used in algal, chronic *Daphnia* and chronic fish tests did not allow for dissipation of the compound that would occur under more realistic exposure conditions, and therefore the notifier proposed that the chronic assessments for fish and invertebrates should be based on data from 34-day and 21-day flow-through studies, combined with time-weighted average (TWA) PEC_{sw} values.

In an additional submission in May 2006, the notifier provided further justification for using the time weighted average PEC_{sw} for the risk assessment for fish and aquatic invertebrates. A summary of the argumentation is given below.

In the case of the fish early life stage test the notifier proposed that the study duration is an appropriate TWA for the following reasons:

- The most sensitive endpoint was growth. Throughout the life stage exposed, from egg to larval fish; 34-days post fertilisation, growth is continuous.
- There is no indication that the effect on growth results from a specific mode of action other than general or systemic toxicity.
- The fish early life stage is generally considered to be the most sensitive in relation to other portions of the lifecycle (McKim, 1985[10]) (in terms of general toxicity).
- A later study (Surprenant, 1990), of longer duration also found a significant effect on growth at 30 days post hatch (LOEC = 19 µg difenoconazole/L). As this test was originally initiated as a fish full lifecycle study exposure was continued until 60 days post hatch. Importantly at 60 days post hatch there was no longer a significant effect on fish growth (or any other endpoint). Thus indicating that 0 to *ca* 30 days exposure is a sensitive 'window' for chronic fish exposure to difenoconazole.

[10] *McKim, J., 1985. Early lifestage toxicity tests. In: G. Rand and S. Petrocelli (Editors), Fundamnetals of Aquatic Toxicology: Methods and applications. Hemisphere, New York, pp. 58-95.*

- The fish bioaccumulation potential of difenoconazole is low with a whole body bioconcentration factor (BCF) of around 330 (Forbis, 1987). Depuration is also rapid, with residue levels in fish tissues declining rapidly when there is no longer exposure to the chemical. Coupled with the rapid dissipation of difenoconazole from the water phase, this indicates that the expected short-term exposures of fish to difenoconazole in surface water are unlikely to lead to long-term systemic exposure of the organisms. This was claimed to provide further reassurance that the NOECs from the laboratory studies, where exposure is maintained, are likely to be conservative.

Consequently it was concluded by the notifier that it would not be unrealistic to apply a TWA approach in conducting the chronic risk assessment. Re-calculated TER values using 28 days TWA and, as a more conservative approach, 7 days TWA is given in the table below.

Table B.9.2.9-9: TER values based on maximum TWA PECs from FOCUS Step 3. Notifier's proposal.

TWA	Endpoint	Value (µg ai/L)	Pome fruit		Carrots	
			PEC (µg ai/L)	TER	PEC (µg ai/L)	TER
28-days	Fish growth 34-d NOEC	7.6	0.191	40	0.085	89
7-days	Fish growth 34-d NOEC	7.6	0.326	23	0.190	40

In case of aquatic invertebrates, the notifier proposed that a time to effect could be delineated from the study as reproduction is in discrete broods. At the NOEC level (5.6 µg difenoconazole/L) the first brood contained *ca* 50% less neonates than the water and solvent control broods. After the first brood recovery in reproductive output was noted and thereafter the number of neonates was comparable to the controls. Therefore, the notifier proposed that a 7-day TWA is appropriate as this coincides with this transitional effect noted in the study.

The re-calculated TER values for pome fruit and carrots based on 7 days TWA are given in the table below.

Table B.9.2.9-10: TER values based on maximum TWA PECs from FOCUS Step 3

TWA	Endpoint	Value (µg ai/L)	Pome fruit		Carrots	
			PEC (µg ai/L)	TER	PEC (µg ai/L)	TER
7-day	Invertebrate reproduction 21-d NOEC	5.6	0.326	17	0.190	29

From the RMS point of view, the available chronic studies on fish and invertebrates do not give sufficient information on the exposure time needed for the onset of the observed effects. The chronic risk assessment for fish was based on the fish early life stage (ELS) study (**Surprenant, 1987b**), where NOEC was derived from effects on larval weight that was recorded only at the end of the study after 34 days exposure. For invertebrates, the assessment was based on number of young per adult in a 21 day study (**Forbis, 1988b**) where effects were clearly observed at an early stage of the test period. Therefore, since the relevant exposure time window for reproductive effects is unknown, it is the RMS opinion that TER values should be based on maximum PEC_{sw} values. The use of global maximum or time weighted average PEC_{sw} for the risk assessment needs to be further discussed.

Since the RMS was not convinced that time weighted average PECsw values should be used for refinement, also FOCUS Step 4 calculation was required in order to establish appropriate risk mitigation levels. These were submitted in May 2006. Recalculated TER values based on the global maximum values for each scenario are given in the table below.

Table B.9.2.9-11: Refined aquatic risk assessment using Step 4 FOCUS modelling. Carrots, 3 x 125 g as/ha, 14 days interval between treatments (covers also pome fruit in Northern EU, 4 x 56.25 g as/ha, 7 days interval between treatments).

Test substance	Scenario[1]	Water body type	Test organism	Time scale	Toxicity end point (µg/L)	Distance (m)	PEC_{sw} global max. (µg/L)	TER	Annex VI trigger
difenoconazole	D3	ditch	*Daphnia magna*	long term	5.6	5	0.151	37	10
difenoconazole	D6	ditch	*Daphnia magna*	long term	5.6	5	0.150	37	10
difenoconazole	R1	pond	*Daphnia magna*	long term	5.6	5	0.044	127	10
difenoconazole	R1	stream	*Daphnia magna*	long term	5.6	5	0.162	35	10
difenoconazole	R2	stream	*Daphnia magna*	long term	5.6	5	0.180	31	10
difenoconazole	R3	stream	*Daphnia magna*	long term	5.6	5	0.206	27	10
difenoconazole	R4	stream	*Daphnia magna*	long term	5.6	5	0.392	14	10

[1] *drainage (D1-D6) and run-off (R1-R4)*

Table B.9.2.9-12: Refined aquatic risk assessment using Step 4 FOCUS modelling. Pome fruit Southern EU, 4 x 75 g as/ha, 7 days interval between treatments

Test substance	Scenario[1]	Water body type	Test organism	Time scale	Toxicity end point (µg/L)	Distance (m)	PEC_{sw} global max. (µg/L)	TER	Annex VI trigger
difenoconazole	D3	ditch	*Daphnia magna*	long term	5.6	14	0.326	17	10
difenoconazole	D3	ditch	*Daphnia magna*	long term	5.6	20	0.325	17	10
difenoconazole	D4	pond	*Daphnia magna*	long term	5.6	14	0.101	55	10
difenoconazole	D4	pond	*Daphnia magna*	long term	5.6	20	0.064	88	10
difenoconazole	D4	stream	*Daphnia magna*	long term	5.6	14	0.351	16	10
difenoconazole	D4	stream	*Daphnia magna*	long term	5.6	20	0.183	31	10
difenoconazole	D5	pond	*Daphnia magna*	long term	5.6	14	0.101	55	10
difenoconazole	D5	pond	*Daphnia magna*	long term	5.6	20	0.064	88	10
difenoconazole	D5	stream	*Daphnia magna*	long term	5.6	14	0.378	15	10
difenoconazole	D5	stream	*Daphnia magna*	long term	5.6	20	0.197	28	10
difenoconazole	R1	pond	*Daphnia magna*	long term	5.6	14	0.095	59	10
difenoconazole	R1	pond	*Daphnia magna*	long term	5.6	20	0.067	84	10
difenoconazole	R1	stream	*Daphnia magna*	long term	5.6	14	0.287	20	10
difenoconazole	R1	stream	*Daphnia*	long	5.6	20	0.230	24	10

Test substance	Scenario[1]	Water body type	Test organism	Time scale	Toxicity end point (µg/L)	Distance (m)	PEC_{sw} global max. (µg/L)	TER	Annex VI trigger
			magna	term					
difenoconazole	R2	stream	*Daphnia magna*	long term	5.6	14	0.381	15	10
difenoconazole	R2	stream	*Daphnia magna*	long term	5.6	20	0.198	28	10
difenoconazole	R3	stream	*Daphnia magna*	long term	5.6	14	0.407	14	10
difenoconazole	R3	stream	*Daphnia magna*	long term	5.6	20	0.292	19	10
difenoconazole	R4	stream	*Daphnia magna*	long term	5.6	14	0.444	13	10
difenoconazole	R4	stream	*Daphnia magna*	long term	5.6	20	0.444	13	10

[1] *drainage (D1-D6) and run-off (R1-R4)*

Based on the maximum PEC_{sw} calculated according to FOCUS Step 4 scenarios including risk mitigation measures, all long term TER values were above the trigger of 10. In conclusion, no further refinement is needed.

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

B.9.3.1 Acute oral and long term toxicity

Details of available mammalian toxicity studies with difenoconazole and the plant metabolite CGA 131013 are provided in **Document M-III, Section 3** and in Section B.6 of this DAR. A summary of the endpoints used in the notifier's risk assessment is presented in the table below.

Table B.9.3.1-1: Toxicity of difenoconazole and the plant metabolite CGA 131013 to mammals

Study Type	Species	End-point	Value	Reference
Difenoconazole				
Acute oral toxicity	Rat	LD_{50}	1453 mg as/kg bw	Argus *et al* (1987)
2-generation reproduction	Rat	NOAEL	17.3 mg as/kg bw/day	Giknis (1988)
CGA 131013				
Acute oral toxicity	Rat	LD_{50}	>5000 mg as/kg bw	Mihail, F. (1982)
Developmental	Rat	NOAEL	100 mg as/kg bw/day	Clapp *et al*., 1983

The RMS agrees with the proposed endpoints for the acute and long term assessment. In the 2-generation study with difenoconazole on rats, there was a slight (7%) but statistically significant effect on the mean pup weight of males in the F1 generation on day 21 at 17.3 mg as/kg bw per day, but this was not considered to be biologically or ecologically significant. No effects were seen on the F2 generation.

The available studies are considered to fulfil the data requirements of Annex II and III of 91/414, and are considered to be sufficient for the risk assessment for wild mammals.

B.9.3.2 Risk assessment for mammals

B.9.3.2.1 SEED TREATMENT WITH DIVIDEND 030FS

B.9.3.2.1.1 First tier risk assessment

A risk assessment for wild mammals at the use of difenoconazole for seed treatment of wheat with the formulation DIVIDEND 030FS was provided by the Notifier in Document M-III, section 6. Based on comments and proposals from the RMS, the risk assessment was amended in an additional submission in January 2006. A summary of the risk assessment is given below.

According to the notifier, treated seeds are incorporated into the soil with a seed drill at depths of 2 cm or more and therefore, are not widely available for consumption by wild mammals. Exposure is only considered likely to occur following occasional, accidental spillages and as a result of seed remaining on the soil surface when the drill lifts and turns. When seed does remain on the soil surface, the seed treatment is assumed to dissipate rapidly by dissolution in rain, dew or soil water. Further, as winter wheat seed is typically expected to germinate within 7 days of sowing, treated-seed will only be available for consumption for a short period. Therefore, it was proposed by the RMS that exposure via seed could be limited to 7 days after sowing for the long term assessment.

ETE values were calculated for the standardised realistic worst-case scenario recommended in the **EU Guidance Document (SANCO/4145/2000)** for seed-treatment, i.e. 25 g mouse. ETE values were calculated according to the following equation:

$$\text{ETE (mg ai/kg bw/day)} = \frac{\text{FIR}}{\text{bw}} \times \text{C} \times \text{AV} \times \text{DHF} \times \text{PD} \times \text{PT}$$

Where:

FIR	Food intake rate of indicator species (g fresh weight/day)
bw	Bodyweight (g)
C	Concentration of compound in fresh diet (mg as/kg seed)
AV	Avoidance factor (1 = no avoidance, worst case; 0 = complete avoidance)
DHF	De-husking factor = 1
PT	Proportion of diet obtained in treated area (1 = worst-case)
PD	Proportion of food type in diet (1 = worst-case)

For the purpose of the first tier risk assessments, it was assumed that there would be no de-husking or avoidance, that mammals obtained 100% of their diet within the treated area and that difenoconazole treated-seed represented 100% of the diet. Therefore, the factors AV, DHF, PT and PD were assumed to be 1. ETE values were calculated for a small, granivorous mammal such as a mouse weighing 25g.

As difenoconazole is systemic, **herbivorous mammals** may be exposed by the consumption of residues in plant tissues grown from seeds treated with DIVIDEND 030FS. According to the notifier, a significant proportion of active ingredient is likely to remain on the seed coat, be lost into soil or taken up into root tissue that will not be available for consumption by mammals. However, for acute and short-term risk assessments, it was assumed that shoots are consumed by a herbivorous mammal, such as a vole, which weighs 25 g. The notifier's assessment was based on a small herbivorous mammal, such as a field vole (standard species according to SANCO 4145/2000). However, it is known that such small mammals are rare visitors on open fields with no vegetation cover, since they want to avoid predators. Therefore, the RMS proposed to include also a larger mammal (ie. rabbit or hare) as focal species for the risk assessment. Acute and short-term ETE values were calculated by assuming that 100% of compound present on each seed is taken up into a rapidly-growing wheat shoot that is twice the weight of the seed. For the long term assessment, a shoot weight of 6 times the treated seed was assumed.

In the assessment of the metabolite, the maximum of 60% of the estimated amount of parent substance was assumed. The same value was also used in the assessment for spray application. However, no data was presented to support this assumption. Therefore, the RMS proposes as a worst case assumption that 100% of the parent compound is transformed to the metabolite (correction for molecular weight, 156 g/mole, compared to 406 g/mole for the parent, or a factor of 0.38 will be taken into account). Based on effect data for the metabolite, an acute oral LD_{50} value of >5000 mg/kg bw and a long term NOAEL value of 100 mg/kg bw per day will be used.

The ETE was then estimated as follows:

$$\mathbf{ETE\ (mg\ ai/kg\ bw/day)} = \frac{\mathbf{FIR}}{\mathbf{bw}} \times \frac{\mathbf{C}}{\mathbf{G}} \times \mathbf{PD} \times \mathbf{PT}$$

Where:

FIR	Food intake rate of indicator species (g fresh weight/day)
bw	Bodyweight (g)
C	Concentration of compound on seed (mg as/kg seed)
G	Growth factor i.e. ratio of shoot to seed weight
PT	Proportion of diet obtained in treated area (1 = worst-case)
PD	Proportion of food type in diet (1 = worst-case)

For the purpose of the first tier risk assessments, it was assumed that mammals obtained 100% of their diet within the treated area and that wheat shoots from difenoconazole-treated seed represented 100% of the diet. Therefore, the factors PT and PD were assumed to be 1. The ETE values for the metabolite CGA 131013 was assumed to be 100% of that estimated for the parent difenoconazole, with correction for molecular weight (factor 0.38).In accordance with EU Guidance Document on Risk Assessment for Birds and Mammals (SANCO/4145/2000), substances with a log P_{ow} greater than 3 have potential for bioaccumulation and should

also be assessed for the risk of biomagnification in terrestrial foodchains. Therefore, the risk of difenoconazole (log P_{ow} of 4.4) to earthworm-eating, fish-eating and predatory mammals was assessed by the notifier.

The following equation was used to assess the potential risk to **mammals feeding on earthworms** containing difenoconazole residues.

$$TER = \frac{NOEL\ (mg/kg)}{PEC_{worm}\ (mg/kg)^{1}\ x\ 1.4^{2}}$$

[1] $PEC_{worm} = PEC_{soil}$ (3 week twa) * BCF where $BCF = C_{worm}/C_{soil}$ i.e.$(0.84 + 0.01\ K_{ow}) / f_{oc} * K_{oc}$

[2] 1.4 is a constant used to convert the PEC_{worm} to a daily dose and is based on a 10g mammal eating 14 g worms per day (Crocker *et al.* 2002).

The PEC_{worm} was calculated as indicated above, but also using a BCF estimated from an earthworm bioaccumulation study. The resulting TER values are presented in the table below. The resulting TERs are greater than the long-term trigger value of 5, indicating that negligible long-term risk to wild mammals feeding on earthworms exposed to DIVIDEND 030FS. Since the RMS considers that the earthworm bioaccumulation study was not valid, the estimated BCF is regarded as the most reliable and should be used in the risk assessment.

With regard to the metabolites formed in soil, CGA 71019 (max ca 23%) has a Log P_{ow} of -1, and therefore no secondary exposure via soil organisms is anticipated. One metabolite however, CGA 205375, was formed at a maximum level of around 10% and has a Log P_{ow} of 3.8. Therefore, the potential for secondary poisoning should be addressed. No data on long term toxicity to mammals is available. However, given that the maximum PECsoil is ca 1/10 of that for difenoconazole, the metabolite would need to be >400 times more toxic than the parent compound for causing a concern (TER trigger 5) for secondary poisoning via earthworms. Hence no further data is considered necessary.

Table B.9.3.2-1: Long-term risk to mammals from secondary poisoning by feeding on earthworms after seed treatment with DIVIDEND 030FS, calculated by the notifier.

Parameter	Estimated BCF[(2)]	BCF from earthworm study[(3)]
PEC_{soil} (mg as/kg)[(1)]	0.016	0.016
BCF	3.35[2]	<1.0[3]
PEC_{worm} (mg as/kg)	0.054	0.016
NOAEL mg as/kg/day	17.3	17.3
TER	229	772

[(1)] maximum predicted difenoconazole concentration in soil following final application assuming no foliar interception
[(2)] $BCF = C_{worm}/C_{soil} = (0.84 + 0.01\ K_{ow}) / f_{oc} * K_{oc}$ (K_{ow} =25118; f_{oc} =0.02; K_{oc} =3759)
[(3)] estimated from earthworm bioaccumulation study (Van der Kolk, 2001)

The following equation was used to assess the potential risk to **wild mammals feeding on fish** containing difenoconazole residues.

$$TER = \frac{NOEL\ (mg/kg)}{PEC_{fish}\ (mg/kg)^{1}\ x\ 0.13^{2}}$$

[1] $PEC_{fish} = PEC_{water} * BCF$

[2] 0.13 is a constant used to convert the PEC_{fish} to a daily dose and is based on a 3000 g mammal eating 346 g fish per day (Crocker *et al.* 2001).

PEC_{fish} and TER values provided by the notifier are presented in the table below. The resulting TERs are greater than the long-term trigger value of 5, indicating that negligible long-term risk to wild mammals feeding on fish exposed to difenoconazole.

With regard to metabolites in water sediment studies, CGA 205375 was formed at a maximum level of around 12% and has a Log P_{ow} of 3.8. However, based on the slightly lower Log Pow value compared to the parent, and assuming that the metabolite is not significantly more toxic than the parent (ca 1200x), the risk for secondary poisoning via fish is considered to be low.

Table B.9.3.2-2: Long-term risk to wild mammals from secondary poisoning by feeding on fish after seed treatment with DIVIDEND 030FS. Corrected according to RMS evaluation in Annex B.8.

Parameter	Value
PECsw (µg as/L)(1)	0.69
BCF(2)	320
PEC_{fish} (mg as/kg)	0.22
NOAEL (mg as/kg bw/day)	17.3
TER	604

(1) maximum predicted difenoconazole concentration in water at 3 m based on FOCUS Step 1).
(2) BCF from bioaccumulation study in bluegill sunfish (Forbis, 1987).

Results from adsorption, distribution, metabolism and excretion (ADME) studies indicate a low bioaccumulation potential, as difenoconazole is extensively metabolised and almost completely eliminated within 7 days of exposure (**Document MIII, Section 3**). Therefore, the risks from **secondary exposure and bioaccumulation** are expected to be low.

The risk assessment for **exposure via drinking water** was carried out in accordance with the SANCO/4145/2000 guidance. The concentration in drinking water that mammals may be exposed to was considered to be equal to the PEC_{sw}. It was not considered that wild mammals would be exposed via drinking water in the field following the representative use of difenoconazole for seed treatment.

Hence the PEC drinking water was assumed to be 0.69 µg as/L (based on FOCUS Step 1 simulations) and the total water ingestion rate for a small mammal (25 g) was calculated as $0.099*bw^{0.99}$ =0.0026 L/day. The daily dose of difenoconazole was calculated as PECdrinking water*total water ingestion rate/bw (0.00069*0.0026/0.025) which was compared to the long term NOEL of 17.3 mg as/kg bw day, resulting in a TER >200000 indicating a low risk to mammals from exposure via drinking water.

The first tier TER values of difenoconazole and the plant metabolite CGA 131013 for granivorous and herbivorous mammals based on the comments given above are summarised in the table below. The animals are assumed to feed solely on the treated field.

Table B.9.3.2-3: First tier risk assessment for mammals at the representative use of difenoconazole in wheat seeds.

Organism	Category	Time frame parent metabolite	FIR /bw (kg fw food/kg bw day)	C (mg as/kg seed)	MAF	f_{twa}	ETE (mg as/kg bw/d)	Tox value (mg as/kg bw/d)	TER
Seed treatment, 60 mg as/kg seed.									
Granivorous mammal	seeds	Acute	0.23	60	1	1	13.80	1453	105
		metabolite		23***			5.24	>5000	954
		Long-term	0.23	60	1	0.33	4.54	17.3	**3.8**
		metabolite		23***			1.73	100	58
Herbivorous mammal	young shoots	Acute*	1.39	60	1	1	83.40	1453	17
		metabolite		23***			31.69	>5000	158
		Acute**	0.28	60			16.80	1453	87
		metabolite		23***			6.44	>5000	776
		Long-term*	1.39	60	1	0.17	14.18	17.3	**1.2**
		metabolite		23***			5.39	100	19
		Long-term**	0.28	60	1	0.17	2.86	17.3	6.1
		metabolite		23***			1.09	100	92
Earthworm eater	Earthworms	Long term	1.4	0.054	1	1	0.076	17.3	229
Fish eater	Fish	Long term	0.13	0.218	1	1	0.028	17.3	610

**First tier based on default small herbivorous mammal*
***Higher tier based on more realistic medium sized herbivorous mammal*
****C assuming that the metabolite accounts for 100% of the parent, with correction for molecular weight by a factor of 0.38.*

In conclusion, the acute TER values were above the trigger of 10 in Annex VI of 91/414, and the refined long term TER for herbivorous mammals was above the trigger of 5. However, for the granivorous mammal, the long term TER values were below the trigger of 5, and therefore a refined assessment is needed for this scenario.

B.9.3.2.1.2 Refined assessment for mammals at seed treatment with DIVIDEND 030FS.

Granivorous mammals

In an additional submission in January 2006, the notifier has provided a refined assessment for granivorous mammals at seed treatment with Dividend. A summary is given below.

The first tier risk assessment assumed that a granivorous mammal (mouse) obtains all of its' dietary requirements by feeding exclusively upon treated seed i.e. PT and PD =1. The typical granivorous mammal for consideration in risk assessment is the wood mouse (*Apodemus sylvaticus*). **Barber et al (2003[11])** investigated exposure of small mammals especially wood mice to pesticide treated wheat seed by analysing stomach contents of wood mice trapped in and around newly-sown wheat fields. This work showed that 90% of the mice had less than 20% (by volume) wheat seed in the stomach and the highest volume recorded was 40%. The diet of the

[11] *Barber I, Tarrant KA & Thompson HM (2003): Exposure of small mammals, in particular the wood mouse Apodemus sylvaticus, to pesticide seed treatments. Environmental Toxicology and Chemistry, Vol 22, No. 5, 1134-1139.*

wood mouse has also been investigated by **Pelz (1989),** as cited by **Gurney et al. (1998)[12],** for arable farmland in Germany. The highest consumption of cereals seeds recorded in this study was 53% in January. **Green (1979[13])** also investigated wood mouse diet and found the highest consumption of cereal seeds for wood mice in a winter wheat field was 60% in the autumn. Therefore, as a worst-case, a refined risk assessment will be conducted using the diet recorded by **Green (1979)** for wood mouse given below.

Table B.9.3.2-4: Proportions of different food types in wood mouse diet in winter wheat, September to December (Green, 1979).

Food	% stomach contents by volume
Cereal seeds	60
Weed seeds	24
Arthropods	16

Based on a DEE of 67.83 kJ for the wood mouse (calculated based on the Guidance Document SANCO 4145/2000, equation "mammals", body weight 25 g), and the energetic content of food items consumed by the wood mouse (Appendix I, Table 3 of the Guidance Document, the daily consumption of the different diet components was calculated. The diet proportions expressed as % volume were considered to equate to proportions expressed as wet weight. Calculation steps and resulting data are shown in the table below.

Table B.9.3.2-5: Calculation of daily consumption of different diet components for the wood mouse

Food type	Energetic content of food [a)]	Assimilation efficiency [b)]	Energetic content of food, weighted by assimilation efficiency	Proportion of different food items in diet mix	Energy uptake per gram of diet mix [c)]	DEE	Daily food consumption [d)]
	(kJ/g wet wt)	(%)	(kJ/g wet wt)	(% of diet wet weight)	(kJ/g dry wt)	(kJ)	(g dry wt/day)
Cereal seeds	14.99	83	12.44	60	7.46		3.36
Weed seeds	18.63	83	15.46	24	3.71		1.34
Large Arthropods	6.71	88	5.90	16	0.94		0.90
Total	-	-	-	100	12.11	67.83	5.60

[a)]Taken from Appendix I, Table 3 of the Guidance Document on Risk Assessment for Birds and Mammals
[b]Taken from Appendix I, Table 5 of the Guidance Document on Risk Assessment for Birds and Mammals
[c)]Calculated as Energetic content of food, weighted by assimilation efficiency x portion of different food items in diet mix/100
[d)]Calculated as (DEE ÷ Total energy uptake per gram of diet) x Portion of different food items in diet mix/100

Since DIVIDEND is applied as a seed treatment, it is reasonable to assume that the weed seed and arthropod components of the wood mouse diet will not contribute any residues of difenoconazole. Therefore, a refined risk assessment was conducted using the daily intake rate for cereal seeds.

[12] Gurney JE, Perrett J, Crocker DR & Pascual JA (1998) "Mammal bible". Mammals and farming: information for risk assessment. November 1998 update. Central Science Laboratory; Project No. M37. Milestone Report. [Citing data from: Pelz HJ (1989) Ecological Aspects of damage to sugar beet seeds by Apodemus sylvaticus. Pp 34-48 in 'Mammals as pests' (ed. RJ Putman), Chapman & Hall, London. The data from Pelz (1989), for woodmouse on arable land, was considered to be the most applicable to the current risk assessment.] Available at http://www.pesticides.gov.uk/approvals.asp?id=1183
[13] Green R (1979) The ecology of wood mice (Apodemus sylvaticus) on arable farmland. J.Zool. 118: 357-377.

Table B.9.3.2-6: Refined long-term risk assessment for wood mouse consuming a mixed diet. Exposure assumed to be limited to one week after sowing, initial concentrations. Ftwa 0.33 to account for a time window of 21 days.

Type of mammal	FIR/bw (g fw/g bw/day)	Seed treatment rate (mg as/kg seed)	f_{TWA}	Difenoconazole ETE (mg as/kg bw/day)	TER_{LT}
Mouse (25 g)	0.13	60	0.33	2.57	6.7

The refined long-term TER value for wood mouse proposed by the notifier is 6.7 which is above the trigger value of 5 and indicates acceptable long-term risk to granivorous mammals from DIVIDEND-treated seed. This risk assessment is still conservative since no allowance has been made for dissipation of difenoconazole residues on seeds due to weathering and other dissipation processes.

RMS comments on the refined risk assessment for granivorous mammals at the use of DIVIDEND 030FS.

The RMS agrees with the risk assessment approach proposed by the notifier. A number of studies on the dietary composition of wood mouse were cited, and the highest proportion of cereal seeds in the diet was selected from available data. Hence, no further refinement is considered necessary for granivorous mammals.

Herbivorous mammals

In an additional submission in January 2006, the notifier has provided additional data and refined assessment for herbivorous birds and mammals at seed treatment with DIVIDEND. A summary of the studies is given in section B.9.1.5.

RMS comments on the refined long term risk assessment for herbivorous mammals at the use of DIVIDEND 030FS.

The RMS generally agrees with the proposed approach for risk assessment for herbivorous mammals provided by the notifier. Regarding the shoot weight data, a time weighted average approach is used by the RMS, compared to the point estimate on day 7 as proposed by the notifier. The refined long term risk assessment for herbivorous mammals is given in the table below. For the plant metabolite CGA 131013, as a conservative approach, it is assumed that an equal amount as for the parent (on a molar basis) is present in the shoot.

Table B.9.3.2-7: Refined long term TER values for herbivorous mammals for difenoconazole and the plant metabolite CGA 131013 after treatment of wheat seeds at a rate of 60 mg difenoconazole/kg seed, based on data on systemicity of radiolabelled difenoconazole in wheat shoots and on seedling weight at different time points after emergence.

Organism	Category	Time frame	FIR /bw (kg fw food/kg bw day)	C* (mg as/kg)	MAF	f_{twa}	ETE (mg as/kg bw/d)	NOAEL (mg as/kg bw/d)	TER
Small sized herbivorous mammal	young shoots	Long-term	1.39	0.72	1	0.17	0.17	17.3	102
		metabolite	1.39	0.27	1	0.17	0.063	100	1587
Medium sized herbivorous mammal	young shoots	Long-term	0.28	0.72	1	0.17	0.034	17.3	509
		metabolite	0.28	0.27	1	0.17	0.013	100	7781

**calculated as 1.2% of the treatment rate for difenoconazole, metabolite assumed to account for 100% of the parent, with correction for molecular weight by a factor of 0.38.*

The refined long term TER values for herbivorous mammals feeding on shoots from treated wheat seeds were all above the trigger of 5 for difenoconazole and the plant metabolite CGA 131013, and no further refinement is needed.

B.9.3.2.2 SPRAY APPLICATION WITH SCORE 250EC

B.9.3.2.2.1 First tier risk assessment

A risk assessment for mammals at dietary exposure following spray application of difenoconazole to pome fruit and carrots using the formulation SCORE 250EC was provided in Document M-III, section 6. Based on comments and proposals from the RMS, the risk assessment was amended in an additional submission in January 2006. A summary of the first tier risk assessment is given below.

The exposure of wild mammals to difenoconazole was assumed to be predominantly dietary, through the consumption of dry residues on food items. Exposure to difenoconazole *via* dermal and inhalation routes is considered unlikely, since at the time of application and for a short period thereafter, the notifier stated that most wild mammals will leave the immediate vicinity of spray operations in response to the human disturbance.

The Estimated Theoretical Exposure (ETE) values to difenoconazole for appropriate scenarios were estimated according to **EU Guidance Document SANCO/4145/2000**, based on the maximum use rates of 4 applications of 75 g as/ha at 7-day intervals in pome fruit and 3 applications of 125 g as/ha at 14 day intervals in carrots. Maximum ETE values were calculated for use in assessing the short-term risk. Time-weighted average ETE values were calculated for use in assessing long-term risks. ETE values were calculated by the notifier using the following equation:

$$\mathbf{ETE\ (mg\ ai/kg\ bw/day)} = \frac{\mathbf{FIR}}{\mathbf{bw}} \times \mathbf{RUD} \times \mathbf{Application\ rate} \times \mathbf{MAF} \times \mathbf{f_{TWA}}$$

where FIR = Food Intake Rate (g fresh weight per day)
bw = Body weight (g)
RUD = Residue per unit dose (mg/kg fresh weight)
MAF = Multiple Application Factor
f_{TWA} = Time-weighted average factor (only used for calculating long-term ETE)

As recommended by SANCO 4145/2000, the 90th percentile residues on food items were used for the acute risk assessment, and the 50th percentiles for the long-term risk assessment. For assessing acute exposure, special MAF_{90FI} values were used, as given in the Guidance Document. For assessing long-term exposure MAF values were calculated from the following equation:

$$MAF = (1-e^{-nki})/(1-e^{-ki})$$

The f_{TWA} was calculated from the following equation:

$$f_{TWA} = (1-e^{-kt})/kt$$

where n = number of applications
k = ln2/DT50
i = interval between applications
t = averaging time (21 days or spray interval between applications if less)

A measured foliar half-life of 7.7 days, claimed to represent the ninetieth percentile (worst case) DT_{50} for difenoconazole in foliage from studies in leek and lettuce (**Walser, 2001**), was used by the notifier in the calculations. However, the RMS considered that these data are not representative for the proposed use of difenoconazole in orchards and carrots. Hence, the RMS maintained the default DT_{50} of 10 days and the corresponding f_{twa} and MAF in plant material. As recommended in the Guidance Document, , a time equivalent to the application interval was used to calculate f_{TWA} in order to ensure that the maximum TWA residue was not underestimated.

The first tier ETE values were calculated by the notifier for the following scenarios: (1) orchard crop, i.e. small herbivorous mammal consuming short grass (FIR/bw 1.39) and (2) leafy crops, i.e. medium herbivorous mammal consuming leafy crops (FIR/bw 0.28). For orchard crops, the Guidance Document SANCO 4145/2000 assumes that for a fungicide such as SCORE 250EC 60% of the applied amount will reach ground vegetation due to interception by the crop (pome fruit, medium crop cover). In the first tier risk assessment, this was allowed for by using correspondingly reduced RUD values.

For the metabolite, the notifier proposed to assume a maximum of 60% in plant material. This value was not fully justified, since the available residue data was not derived from studies on carrots or grass, and also due to the fact that the number and timing of samples taken was not considered as sufficient to establish a reliable maximum value. Hence, the RMS proposed to assume as a worst case that 100% of the parent compound will be transformed to the metabolite. The ETE was corrected for molecular weight, which was a factor of 0.38 lower than that of the parent (156:406).

In accordance with EU Guidance Document (SANCO/4145/2000), substances with a log P_{ow} greater than 3 have potential for bioaccumulation and should be assessed for the risk of **biomagnification in terrestrial foodchains**. Therefore, the risk of difenoconazole (log P_{ow} of 4.4) to earthworm-eating, fish-eating and predatory mammals has been assessed.

The following equation was used to assess the potential risk to **mammals feeding on earthworms** containing difenoconazole residues.

$$TER = \frac{NOEL\ (mg/kg)}{PEC_{worm}\ (mg/kg)^{(1)} \times 1.4^{(2)}}$$

(1) $PEC_{worm} = PEC_{soil}$ (3 week twa) * BCF where $BCF = C_{worm}/C_{soil}$ i.e.$(0.84 + 0.01\ K_{ow}) / f_{oc} * K_{oc}$

(2) 1.4 is a constant used to convert the PEC_{worm} to a daily dose and is based on a 10g mammal eating 14 g worms per day (Crocker *et al.* 2002).

The PEC_{worm} was calculated by the notifier as indicated above and an additional calculation was performed using a BCF value obtained from an earthworm bioaccumulation study (**Van der Kolk, 2001**) and the maximum soil PEC values following the use of SCORE 250EC in pome fruit and carrots, as given in **Document M-III, Section 5, Point 9.1.2**. The resulting TER values are presented in the table below.

With regard to the metabolites formed in soil, CGA 71019 (max ca 23%) has a Log P_{ow} <0, and therefore no secondary exposure via soil organisms is anticipated. One metabolite however, CGA 205375, was formed at a maximum level of around 10% and has a Log P_{ow} of 3.8. Therefore, the potential for secondary poisoning should be addressed. No data on long term toxicity to mammals is available. However, given that the maximum PECsoil is ca 1/10 of that for difenoconazole, the metabolite would need to be >50 times more toxic than the parent compound for causing a concern for secondary poisoning via earthworms. Hence no further data is considered necessary.

Table B.9.3.2-9: Long-term risk to mammals from secondary poisoning by feeding on earthworms after spray application of SCORE 250EC. PEC_{soil} values corrected according to RMS evaluation in Annex B.8.

Parameter	Pome fruit		Carrots	
	Estimated BCF(2)	BCF from earthworm study(3)	Estimated BCF(2)	BCF from earthworm study(3)
PEC_{soil} (mg as/kg)(1)	0.136	0.136	0.096	0.096
BCF	3.35	1	3.35	1
PEC_{worm} (mg as/kg)	0.46	0.136	0.32	0.096
NOEL (mg as/kg bw/day)	17.3	17.3	17.3	17.3
TER	27	91	39	129

(1) maximum predicted difenoconazole concentration in soil following final application
(2) BCF = $C_{worm}/C_{soil} = (0.84 + 0.01\ K_{ow}) / f_{oc} * K_{oc}$ (K_{ow} =25118; f_{oc} =0.02; K_{oc} =3759)
(3) estimated from earthworm bioaccumulation study (Van der Kolk, 2001, Document M-II, Section 6)assessed as unreliable by RMS.

The following equation was used to assess the potential risk to **wild mammals feeding on fish** containing difenoconazole residues.

$$TER = \frac{NOEL\ (mg/kg\ bw/day)}{PEC_{fish}\ (mg/kg)^{1} \times 0.13^{2}}$$

[1] $PEC_{fish} = PEC_{water} * BCF$

[2] 0.13 is a constant used to convert the PEC_{fish} to a daily dose and is based on a 3000 g mammal eating 346 g fish per day (Crocker *et al.* 2001).

PEC_{fish} and TER values are presented in the table below.

Table B.9.3.2-10: Long-term risk to wild mammals from secondary poisoning by feeding on fish after spray application of SCORE 250EC, calculated by the notifier. Corrected based on RMS evaluation in Annex B.8.

Parameter	Pome fruit	Carrots
PECsw (µg as/L)[1]	4.23	2.73
BCF[2]	320	320
PEC_{fish} (mg as/kg)	1.35	0.87
NOEL (mg as/kg/day)	17.3	17.3
TER	98	152

[1] maximum predicted difenoconazole concentration in water based on FOCUS Step 2.
[2] BCF from bioaccumulation study in bluegill sunfish (Forbis, 1987).

With regard to metabolites in water sediment studies, CGA 205375 was formed at a maximum level of around 12% and has a Log P_{ow} of 3.8. However, based on the slightly lower Log P_{ow} value compared to the parent, and assuming that the metabolite is not significantly more toxic than the parent (ca 140x), the risk for secondary poisoning via fish is considered to be low.

The notifier stated that results from adsorption, distribution, metabolism and excretion (ADME) studies indicate a low bioaccumulation potential, as difenoconazole is extensively metabolised and almost completely eliminated within 7 days of exposure (**Document M-III, Section 3**). Thus, there will be low **secondary exposure or bioaccumulation**, and a low risk to predatory mammals is expected following the application of SCORE 250EC according to use patterns in pome fruit and carrots.

The risk for **exposure via drinking water** was assessed by the RMS in accordance with the SANCO/4145/2000 guidance. The concentration in drinking water that mammals may be exposed to was considered to be equal to the PEC_{sw}. It was not considered that wild mammals following the representative use of difenoconazole in pome fruit and carrots will be exposed through drinking from puddles of spray liquid or from reservoirs held in the axils of leaves.

Hence the PEC drinking water was assumed to be 32.4 µg as/L (based on FOCUS Step 1 simulation for pome fruit) and the total water ingestion rate for a small mammal (25 g) was calculated as $0.099*bw^{0.99}$ =0.0026 L/day. The daily dose of difenoconazole was calculated as PECdrinking water*total water ingestion rate/bw (0.0324*0.0026/0.025) which was compared to the long term NOEL of 17.3 mg as/kg bw day, resulting in a TER >5000 indicating a low risk to mammals from exposure via drinking water.

The a summary of the first tier assumptions made in the risk assessment and the resulting ETE and TER values are given in the table below. The animals were assumed to feed solely on treated field.

Table B.9.3.2-11: First tier risk assessment for wild mammals at the representative use of difenoconazole in carrots and pome fruit. Spray application with SCORE 250EC.

Use patten Organism	Category	Time frame	FIR /bw (kg fw food/kg bw day)	RUD/ C***	MAF	f_{twa}	ETE	Tox value (mg as/kg bw/d)	TER
Carrots, 3 x 125 g as/ha, 14 days interval									
Medium herbivorous mammal	leafy crops	Acute	0.28	87	1.3	1	3.96	1453	367
		metabolite		33**			1.50	>5000	3333
		Long-term	0.28	40	1.5	0.64	1.20	17.3	14
		metabolite		15**			0.46	100	217
Earthworm eater	earthworms	Long term	1.4	0.32***	1	1	0.45	17.3	39
Fish eater	fish	Long term	0.13	0.87***	1	1	0.11	17.3	150
Pome fruit Northern EU, 4 x 56.25 g as/ha, 7 days interval.									
Small herbivorous mammal	short grass	Acute	1.39	85*	1.8	1	7.18	1453	202
		metabolite		32**			2.73	>5000	1831
		Long-term	1.39	46*	2.2	0.79	6.25	17.3	**2.8**
		metabolite		17**			2.34	100	43
Pome fruit Southern EU, 4 x 75 g as/ha, 7 days interval.*									
Small herbivorous mammal	short grass	Acute	1.39	85*	1.8	1	9.57	1453	152
		metabolite		32**			3.64	>5000	1374
		Long-term	1.39	46*	2.2	0.79	8.33	17.3	**2.1**
		metabolite		17**			3.17	100	32
Earthworm eater	earthworms	Long term	1.4	0.45***	1	1	0.63	17.3	28
Fish eater	fish	Long term	0.13	1.87***	1	1	0.24	17.3	71

**crop interception 40% included for intermediate crop cover in orchards according to SANCO/4145/2000.*
*** RUD assuming that the metabolite accounts for 100% of the parent, with correction for molecular weight by a factor of 0.38.*
****PECworm values.*

In the first tier calculations, the acute TER values are above the trigger for all representative use scenarios, and the long term TER values were above the trigger for the use in carrots (parent and metabolite). However, refinement is still needed for the long term toxicity to small herbivorous mammals in pome fruit.

B.9.3.2.2.2 Refined risk assessment for wild mammals at the use of SCORE 250EC in pome fruit

The first tier TER values for orchards were calculated based on the standard assumption that 60% of an insecticide or fungicide will reach short grass growing in the pome fruit crop, as recommended in the Guidance Document. However SCORE 250EC is recommended for early application (equivalent to flowering stage) and for this growth stage only 35% of the application (from FOCUS (2000) is deposited onto short grass growing below the crop. Therefore, the long-term TER for small mammals feeding on short grass was proposed to be refined using this more realistic deposition figure of 35%. This approach was accepted by the RMS.

The notifier also proposed to use the mean DT_{50} value of 5.5 days from residue studies in leek and lettuce (**Walser, 2001**). However, the RMS do not consider these data to be representative for the recommended use of

difenoconazole in pome fruit, and therefore the default value of 10 days from SANCO 4145/2000 will be maintained.

The refined TER values for small herbivorous mammals in pome fruit are given in the table below.

Table B.9.3.2-12: Long-term risk assessment to small herbivorous mammal from difenoconazole applied to pome fruit (DT_{50} 10 days) in northern and southern EU. Refinement based on FOCUS crop interception.

Scenario	Food type	App rate (kg/ha)	FIR/bw	RUD mean	MAF	f_{TWA}	Long-term ETE (mg/kg bw/day)	TER_{LT}
Northern EU								
Small herbivorous mammal	Short grass	0.056	1.39	27*	2.2	0.79	3.67	**4.7**
Southern EU								
Small herbivorous mammal	Short grass	0.075	1.39	27*	2.2	0.79	4.89	**3.5**

**using 65% crop interception*

The refined long-term TER values were 4.7 and 3.5, which is still slightly below the trigger value of 5 and indicate that further refinement is needed for the use of difenoconazole in orchards.

In an additional submission in May 2006, the notifier provided further proposals for refinement based on proportions of different food types in the diet. A summary is given below. Studies of the diet of the common vole, *Microtus arvalis* (bodyweight 25g), have shown that it selectively feeds on certain non-grass plant species e.g. **Rinke (1990[14])** found that for voles on grassland the dicotyledons *Taraxacum officinale* and *Trifolium repens* constituted about 40% of total volume ingested. **Rinke (1991[15])** reported the stomach contents of 363 individual common voles trapped in permanent pasture in Germany and found that dicotyledons represented on average about 63.5% of stomach contents (>80% in more than half of individuals) whereas they represented only about 30% of ground cover in the pasture. Dicotyledons hence seem to be preferentially selected by voles and this needs to be accounted for in the risk assessment for voles feeding in orchards treated with difenoconazole. Based on the above diet references, it was considered reasonable to assume that the diet of a common vole will typically comprise 60% dicotyledons and 40% grasses based upon dry weight, which was proposed to be the closest equivalent to volume of stomach contents.

The typical vegetation within orchards is classified by SANCO/4145/2000 as short grass and it could therefore be assumed that voles will obtain the dicotyledonous component of their diet from outside the treated orchard. However, the ground vegetation in many orchards will contain a mixture of grasses and dicotyledonous plants and hence it is more conservative to assume that a vole obtains all of its diet from within the treated orchards. A

[14] *Rinke, T. 1990. Zur Nahrungsökologie von Microtus arvalis (Pallas, 1778) auf Dauergrünland. I. Allgemeine Nahrungspräferenzen. Zeitschrift für Säugetierkunde 55: 106-114*

[15] *Rinke T. 1991. Percentage of volume versus number of species: availability and intake of grasses and forbs in Microtus arvalis. Folia Zoologica 40 (2):143-151.*

refined risk assessment is conducted below for a vole consuming a mixture of monocotyledonous and dicotyledonous plants in an orchard treated with highest application rate of SCORE.

Table B.9.3.2-13: Calculation of residue concentrations in herbivore food types

Food type	RUD (mean)	Application rate (kg ai/ha)	MAF	f_{TWA}	Inter-ception factor	21-day TWA concentration in food mg/kg fw)
Grasses	76	4 x 0.075	2.2	0.79	65% [a]	3.47
Dicotyledons	40	4 x 0.075	2.2	0.79	65%[a]	1.82

[a] *65% interception by orchard vegetation, i.e. 35% of the application reaches the ground-level vegetation*

The Daily Energy Expenditure (DEE) for the vole is given in SANCO 4145/2000 as 68 kJ/day. Based on the DEE and energy content of food items consumed by the vole (Appendix I, Table 3 of SANCO 4145/2000), the daily consumption of the different diet components was calculated. The dicotyledonous food component of the vole is classified as the food category 'non-grass herbs' in Appendix 1, Table 3 mentioned above, since this category gives the energy content of non-grass weeds (the 'dicot leaves' category refers to energetic content of dicot crops which are not relevant here). Calculation steps and resulting data are shown in the table below.

Table B.9.3.2-14: Calculation of daily consumption of different diet components for the vole

Food type	Energetic content of food [a)]	Assimilation efficiency [b)]	Energetic content of food, weighted by assimilation efficiency	Proportion of different food items in diet mix	Energy uptake per gram of each diet item[c]	DEE	Daily food consumption [d)]
	(kJ/g dry wt)	(%)	(kJ/g dry wt)	(% of diet dry weight)	(kJ/g dry wt)	(kJ)	(g dry wt/day)
Grasses	17.96	46	8.26	40	3.30		2.41
Non-grass herbs	17.98	74	13.31	60	7.99		3.61
Total	-	-	-	100	11.29	68	6.02

[a)] *Taken from Appendix I, Table 3 of the Guidance Document on Risk Assessment for Birds and Mammals*
[b)] *Taken from Appendix I, Table 5 of the Guidance Document on Risk Assessment for Birds and Mammals*
[c)] *Calculated as Energetic content of food, weighted by assimilation efficiency x proportion of different food items in diet mix/100*
[d)] *Calculated as (DEE ÷ Total energy uptake per gram of diet) x Proportion of different food items in diet mix*

The daily consumption of the different diet components given above is based on dry weight. These figures were converted to fresh weight using the moisture content of the different commodities given in SANCO 4145/2000 below. These fresh weight actual food consumption data were then compared to the body weight of the vole (25 g) to give FIR/bw data for each food type. These were multiplied with the estimated residue levels to calculate the ETE for each food type. The ETE values for each food type were then summed to produce an overall ETE for all food items, and the TER was then based on the long-term mammalian NOAEL of 17.3 mg ai/kg bw/day divided by the total ETE. The corresponding calculation steps are shown in the table below.

Table B.9.3.2-15: Calculation of FIR/bw, ETE and TER for a 25 g vole. Refinement of crop interception and diet composition.

Food type	Daily food consumption of different food items (based on DRY weight)	Moisture content [a]	FIR Daily food consumption of different food items (based on FRESH weight)	Body weight of vole	FIR/bw for specific food type	Residue level	ETE	TER_{LT}
	(g dry wt/day)	(%)	(g fresh wt/day)	(g)	(g fresh wt/day/g bw)	(mg ai /kg)	(mg ai /kg bw/day)	-
Grasses	2.41	76.4	10.21	25	0.41	3.47	1.42	
Non-grass herbs	3.61	82.1	20.17		0.81	1.82	1.47	
Total	6.02	-	30.38	-	-	-	2.89	6.0

[a] *Taken from Appendix I, Table 3 of the Guidance Document on Risk Assessment for Birds and Mammals*

$$\text{FIR Daily food consumption (g fw/d)} = \frac{100}{(100 - \text{proportion moisture content})} \times \text{daily food consumption (g dry wt/d)}$$

The refined long-term TER for vole is 6.0 which is above the relevant trigger value of 5 and indicates that no further refinement is needed.

RMS comments on the refined assessment for wild mammals in pome fruit

The RMS agrees with the refinement proposals provided by the notifier.

B.9.3.2.3 RMS overall conclusion on the risk for wild mammals following the representative use of difenoconazole

The acute and short term TER values were above the trigger values for difenoconazole and metabolites in the first tier assessment for all representative use scenarios, and no refinement is needed.

In the long term assessment, the TER values were below the trigger values for difenoconazole following seed treatment with DIVIDEND 030FS and spray applications with SCORE 250EC in pome fruit. However, based on additional data and proposals provided by the notifier and accepted by the RMS, the TER_{lt} values were above the trigger values for all representative use scenarios. Hence, no further refinement is needed.

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

B.9.4.1 Acute toxicity

ACTIVE INGREDIENT

Reference:	**Hoxter, K.A. and Jaber, M. (1989). CGA 169374 an acute contact toxicity study with the honey bee. Wildlife International Ltd., USA. Unpublished report no. 108-302A. (Syngenta File No 169374/0028)**
Guideline:	US EPA FIFRA 141-1.
GLP:	Yes.

Material and methods:

Test substance:	Technical difenoconazole, batch number P-807002, purity 91.1%.
Species:	Honey bee (*Apis mellifera*)
Treatments:	Nominal concentrations 0.013, 0.022, 0.036, 0.06, 0.1 mg/bee. Aliquots (2 µL) of technical difenoconazole stock solutions prepared in acetone were applied to the thorax of the bees. The test also incorporated a solvent control treatment in which bees were treated with 2 µL acetone and an untreated control.
Number of animals:	25 bees in each of 2 replicate test chambers per concentration.
Duration:	48 hours.
Test conditions:	Temperature 21 – 26°C, relative humidity 78%. Darkness except during dosing and observation.
Observations:	Bees were observed for mortality, twice on day 0 and subsequently on days 1 and 2 after dosing.
Data analysis:	Visual inspections.

Results:

The effects of difenoconazole on bee mortality are presented in the table below.

Table B.9.4.1-1: Effect of difenoconazole on bee mortality.

Nominal concentration (mg/bee)	Cumulative mortality (total number out of 50 bees)		
	Day 0	Day 1	Day 2
Control	1	2	2
Control + acetone	1	6	6
0.013	0	0	0
0.022	0	1	1
0.036	0	1	2
0.060	0	2	3
0.100	0	0	1

Observed mortalities were not considered to be related to concentration level and the 48-hour LC_{50} for difenoconazole in honey bees was considered to be >0.1 mg/bee.

RMS comments:

The study was well performed and reported and is considered to be valid for the risk assessment.

Reference:	**Grieg-Smith, P.W. (1990). Acute contact and oral toxicity of CGA 169374 to the honey-bee. ADAS Central Science Laboratory, UK. Unpublished report no. C89/0370. (Syngenta File No 169374/0029)**
Guideline:	Not stated.
GLP:	Yes.

Material and methods:

Test substance:	Technical difenoconazole, batch number P.807002, purity 91.8%.
Species:	Honey bee (*Apis melllifera)*
Treatments:	The acute contact toxicity was investigated by topically dosing bees with aliquots of

	technical difenoconazole prepared in acetone, to give nominal exposure concentrations of 25, 50 or 100 µg/bee. For determination of acute oral toxicity, bees were supplied with a 20% sucrose solution containing difenoconazole prepared in acetone, at nominal concentrations of 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/mL. Both tests incorporated 2 control treatments in which bees were given a topical dose of acetone, for the contact test, or acetone in 20% sucrose for the oral test.
Number of animals:	3 replicate chambers of 10 bees for each concentration level.
Duration:	48 hours.
Test conditions:	Not reported.
Observations:	The weight of the sucrose solution consumed by bees was recorded in the oral study in order to estimate dose per bee. Bees were observed for mortality after 4, 24 and 48 hours.
Data analysis:	Not stated, not needed.

Results:

The effects of difenoconazole on bee mortality are presented in the tables below.

Table B.9.4.1-2: Effect of difenoconazole on bee mortality - Acute contact toxicity test.

Measured concentration (µg/bee)	Cumulative mortality (total number out of 30 bees)		
	4 hours	24 hours	48 hours
Control + acetone	0	0	3
24.9	1	15	15
49.8*	0	3	3
99.7	1	1	3

**6 bees escaped*

Table B.9.4.1-3: Effect of difenoconazole on bee mortality - Acute oral toxicity test.

Nominal concentration (µg/mL)	Average dose per bee (µg/bee)	Cumulative mortality (total number out of 30 bees)		
		4 hours	24 hours	48 hours
Control + acetone	-	0	6	6
100	7.1	1	3	3
200	12.5	0	6	7
400	29.1	0	1	3
810	41.4	1	9	10
1610	80.2	0	5	8
3220	177	0	9	10

Since the observed mortalities did not reach 50%, the 48-hour LC_{50} for contact and oral toxicity of difenoconazole were considered to be >99.7 and >177 µg/bee, respectively, based on the results from the main test.

RMS comments:

Test conditions were not reported. However, since no treatment related effects were observed, the study is considered to support the conclusion that difenoconazole is of low toxicity to bees.

PLANT PROTECTION PRODUCT

DIVIDEND 030FS

No specific study on this formulation was submitted, and is not considered necessary, since the use as seed treatment do not result in exposure of the formulation but only of the active ingredient via systemic action.

SCORE 250EC

Reference:	**Vorwohl, G. (1988). CGD 96430F - Testing toxicity to honeybees in the laboratory. University of Hohenheim, Landesanstalt für Bienenkunde, Hohenheim, D. Unpublished Report No. FA 25/88 (Syngenta No. CGA 169374/0768). Study dates: August – December 1988.**
Guideline:	Not specified; consistent with the precursor to BBA Guideline VI, 23-1 (1991).
GLP:	No. When this study was performed GLP had not been formally adopted; the study was claimed to be performed according to sound scientific practices.
Material and methods:	
Test substance:	CGD 96430F (equivalent to A-7402 A) containing 25% w/v difenoconazole, (CGA 169374).
Species:	Honey bees (*Apis mellifera*)
Treatments:	Adult honeybees were exposed to A-7402 A *via* four routes: residues in air (respiration), contact with dry residues, direct spray and oral toxicity. 1% v/v solutions of A-7402 A were prepared in water for respiration, contact and direct over-spray tests, or in aqueous sucrose for the oral test. Bees were continuously offered aqueous sucrose solution for the test duration and were maintained in test cages as follows: **Respiration test:** Test cages, with perforated bottoms, were placed on Petri dishes half-filled with test solution enabling exposure to residues in the vapour phase. **Contact test:** Test cages were lined with dried filter paper drenched in test solution. **Direct spray test**: Bees were sprayed with test solution using a hand-held sprayer and then transferred to test cages. **Oral test:** Sucrose solutions containing the test substance were offered for 3 hours, and then replaced with untreated sucrose solution for the remaining test period. It was assumed that each bee received 100 µg A-7402 T provided that no repellent effect occurs.
Number of animals:	Each test incorporated 3 replicate cages of 10 bees for the A-7402 A treatment, an untreated control and a toxic standard treatment in which bees were exposed to 0.005% Nexit (active ingredient lindane).
Duration:	24 and 72 hours, respectively.
Test conditions:	Not reported.

Observations: Each experiment was performed twice. On the first occasion, mortality was recorded after 24 hours while on the second occasion mortality was assessed after 24, 48 and 72 hours in respiration, contact and direct-spray tests.

Data analysis: Not stated.

Results:

Honey-bee mortalities in respiration, contact, direct-spray and oral toxicity tests are presented in the table below.

Table B.9.4.1-4: Effect of A-7402 A on mortality in honey bees

Experiment	Treatment	Mortality (%)									
		Respiration			Contact			Direct spray			Oral
		Time after treatment (hours)									
		24	48	72	24	48	72	24	48	72	24
1	Control	0	n.d.	n.d.	10	n.d.	n.d.	13	n.d.	n.d.	0
	A-7402 A	0	n.d.	n.d.	10	n.d.	n.d.	7	n.d.	n.d.	13
	Toxic standard	100	n.d.	n.d.	20	n.d.	n.d.	17	n.d.	n.d.	7
2	Control	7	17	40	3	3	17	3	3	17	3
	A-7402 A	40	40	43	0	n.d.	27	0	37	60	57
	Toxic standard	100	n.a.	n.a.	3	13	27	3	13	27	13

n.d. not determined; n.a. not applicable

The data indicates that the 24-hour acute oral LD_{50} of A-7402 A to honeybees was approximately 100 µg formulation/bee, equivalent to 25 µg as/bee.

RMS comments:

The study report was very scarce (a single data sheet) and written in German, with no information on test conditions and insufficient information on exposure levels. The results will not be used in the further assessment.

Reference: **Anonymous (1989). CGD 96430 F - Testing toxicity to honeybees in the laboratory. Bayrische Landesanstalt für Bienenzucht, Erlangen, D. Unpublished Report No. 4/89 (Syngenta No. CGA 169374/0769). Study dates: January 1989.**

Guideline: Not specified; consistent with the precursor to BBA Guideline VI, 23-1 (1991).

GLP: No. When this study was performed GLP had not been formally adopted; the study was claimed to be performed according to sound scientific practices.

Material and methods:

Test substance: CGD 96430F (equivalenty to A-7402 A) containing 25% w/v difenoconazole (CGA 169374).

Species: Honey bees (*Apis mellifera*)

Treatments: Adult honeybees were exposed to A-7402 A *via* four routes: residues in air (respiration), contact with dry residues, direct spray and oral toxicity. Solutions of A-7402 A were prepared in water for respiration, contact and direct over-spray tests

(1% v/v), or in aqueous sucrose for the oral test (0.1 v/v). Bees were continuously offered aqueous sucrose solution for the test duration and were maintained in test cages as follows:

Respiration test: Test cages, with perforated bottoms, were placed on Petri dishes half-filled with test solution enabling exposure to residues in the vapour phase.

Contact test: Test cages were lined with dried filter paper drenched in test solution.

Direct spray test: Bees were sprayed with test solution using a hand-held sprayer and then transferred to test cages.

Oral test: Sucrose solutions containing the test substance were offered for 3 hours, and then replaced with untreated sucrose solution for the remaining test period. It was assumed that each bee received 10 µg A-7402 A, provided that no repellent effect occurs.

Number of animals: Each test incorporated 3 replicate cages of 10 bees for the A-7402 A treatment, an untreated control and a toxic standard treatment in which bees were exposed to 0.005% Nexit (active ingredient lindane).

Duration: 24 and 72 hours, respectively.

Test conditions: Not reported.

Observations: Mortality was recorded after 24 hours in the oral test and after 24, 48 and 72 hours in respiration, contact and direct-spray tests.

Data analysis: Not stated.

Results:

Honey-bee mortalities in respiration, contact, direct-spray and oral toxicity tests are presented in the table below.

Table B.9.4.1-5: Effect of A-7402 A on mortality in honey bees

Treatment	Mortality (%)									
	Respiration			Contact			Direct spray			Oral
	Time after treatment (hours)									
	24	48	72	24	48	72	24	48	72	24
Control	6	6	6	0	3	3	0	0	0	n.d.
A-7402 A	0	0	0	0	0	0	0	0	0	0
Toxic standard	9	30	30	100	n.a.	n.a.	27	42	42	100*

*n.d. not determined; n.a. not applicable; *assessed after 1 hour.*

The data indicates that the 24-hour acute oral LD_{50} of A-7402 A to honeybees was >10 µg formulation/bee, equivalent to ≥2.5 µg as/bee.

RMS comments:

The study report was very scarce (a single data sheet) and written in German, with no information on test conditions and insufficient information on exposure levels. The results will not be used in the further assessment.

Reference:	**Anonymous (1988). CGD 96430F - Testing toxicity to honeybees in the laboratory. Hessische Landesanstalt für Tierzucht, Abteilung für Bienenzucht, Kirchhain, D. Unpublished Report No. 08/88 (Syngenta No. CGA 169374/0770). Study dates: 7 June – 6 September 1988.**
Guideline:	Not specified; consistent with the precursor to BBA Guideline VI, 23-1 (1991).
GLP:	No. When this study was performed GLP had not been formally adopted; the study was claimed to be performed according to sound scientific practices.

Material and methods:

Test substance:	CGD 96430F (equivalent to A-7402 A) containing 25% w/v of difenoconazole (CGA 169374).
Species:	Honey bees (*Apis mellifera*).
Treatments:	Adult honeybees were exposed to A-7402 A *via* four routes: residues in air (respiration), contact with dry residues, direct spray and oral toxicity. Solutions of A-7402 A (1% v/v) were prepared in water for respiration, contact and direct over-spray tests. In the oral test, A-7402 A was prepared in aqueous sucrose at 0.5 and 1 % v/v. Bees were continuously offered aqueous sucrose solution for the test duration and were maintained in test cages as follows: **Respiration test:** Test cages, with perforated bottoms, were placed on Petri dishes half-filled with test solution enabling exposure to residues in the vapour phase. **Contact test:** Test cages were lined with dried filter paper drenched in test solution. **Direct spray test:** Bees were sprayed with test solution using a hand-held sprayer and then transferred to test cages. **Oral test:** Sucrose solutions containing the test substance were offered for 3 hours, and then replaced with untreated sucrose solution for the remaining test period. It was assumed that each bee received 50 or 100 µg A-7402 A, provided that no repellent effect occurs.
Number of animals:	Each test incorporated 3 replicate cages of 10 bees for the A-7402 A treatment, an untreated control and a toxic standard treatment in which bees were exposed to 0.005% Nexit (active ingredient lindane).
Duration:	24 and 72 hours, respectively.
Test conditions:	Not reported.
Observations:	Mortality was recorded after 24 hours in the oral test and after 24, 48 and 72 hours in respiration, contact and direct-spray tests.
Data analysis:	Not stated.

Results:

Data for honey bee mortality in respiration, contact, direct-spray and oral toxicity tests are presented in the table below.

Table B.9.4.1-6: Effect of A-7402 A on mortality in honey bees

Treatment	Mortality (%)									
	Respiration			Contact			Direct spray			Oral
	Time after treatment (hours)									
	24	48	72	24	48	72	24	48	72	24
Control	0	7	10	0	2	3	0	2	8	0
A-7402 A	0	0	2	0	2	3	0	3	5	52 (1% v/v) 35 (0.5% v/v)
Toxic standard	100	n.a.	n.a.	88	100	n.a.	0	7	8	63

n.a. not applicable

According to the findings, the 24-hour acute oral LD_{50} of A-7402 A to honeybees was approximately 100 µg formulation/bee (25 µg as/bee). For other exposure routes, no significant treatment related effects were observed.

RMS comments:

The study report was very scarce (a single data sheet) and written in German, with no information on test conditions and insufficient information on exposure levels. The results will not be used in the further assessment.

Reference:	**Bew, M.H. (1992). Acute contact and oral toxicity of A-7402 F to the honey-bee. ADAS Central Science Laboratory, UK. Unpublished report no. CC06104. (Syngenta No. CGA 169374/0710). Study dates: 5-12 August 1992.**
Guideline:	Not specified.
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 F containing 250 g/L CGA 169374, batch number P.111003.
Species:	Honey bees (*Apis mellifera*).
Treatments:	Topical dosing of bees with aliquots of A-7402 F prepared in acetone, to give nominal exposure concentrations of 0.1, 1.08, 10.83 and 108.31 µg formulation/bee. For determination of acute oral toxicity, bees were supplied with a 50% sucrose solution containing A-7402 F prepared in acetone, at concentrations of 5, 54, 541 and 5415 µg formulation/mL. Assuming consumption of 20 µL of test solution per bee, these values correspond to 0.1, 1.08, 10.83 and 108.31 µg formulation/bee. The weight of the sucrose solution consumed by bees was recorded in the oral study in order to estimate dose per bee.
Number of animals:	Both tests incorporated 4 replicate chambers of 10 bees for each concentration level and each of 2 control treatments in which bees were given a topical dose of acetone, for the contact test, or acetone in 50% sucrose for the oral test.
Duration:	48 hours.
Test conditions:	Not reported.
Observations:	Bees were observed for mortality after 4, 24 and 48 hours.

Data analysis:	Not stated.

Results:

No mortalities were recorded for control or A-7402 F-exposed bees in the oral or contact toxicity test. Hence, the 48-hour LC_{50} for contact and oral toxicity of A-7402 F were both considered to be >108.31 µg formulation/bee (>27 µg as/bee).

RMS comments:

Test conditions were not reported. Further, this study was conducted with a formulation that is not considered to be equivalent to the representative formulation A-7402 T. Hence, the results will not be used in the further risk assessment.

B.9.4.2 Semi-field studies

Reference:	**Decker, U. (1993). Testing for the toxicity of CGD 96430 F to foraging honey bees, (Apis mellifera L.) under semi-field (tent) conditions. RCC, Fullinsdorf, Switzerland. Unpublished report no. RCC 323436. (Syngenta No. CGA 169374/1272). Study dates: 27 July –15 August 1992.**
Guideline:	BBA VI, 23-1.
GLP:	Yes.
Material and methods:	
Test substance:	CGD 96430F (equivalent to A-7402 A) containing 250 g/L difenoconazole, batch number P.111003.
Species:	Honey bees (*Apis mellifera*).
Treatments:	Beehives were placed into a plot of flowering *Phacelia* and covered with a gauze tent. After 2 days (Trial 1) or 5 days (Trial 2), spray solutions of A-7402 A, prepared in tap water, were sprayed over the crop and foraging bees at a rate of 200 L/ha to give an exposure concentration of 2 L A-7402 A/ha (or 500 g as/ha). The test incorporated three hives for the A-7402 A treatment, three hives for the control, in which crop and bees were sprayed with tap water, and three replicates of a toxic standard in which crop and bees were sprayed with dimethoate (40% formulation) at 1 L/ha. This procedure was repeated in Trial 2 using previously unexposed bees, hives and crop.
Number of animals:	Three hives per treatment level.
Duration:	5 days after treatment.
Test conditions:	Temperature mainly between 20 and 35°C in Trial 1, 15 – 30 in Trial 2. Relative humidity 32 – 100%. The weather was fairly warm and dry during the study.
Observations:	In both trials, bees were monitored daily during acclimatization and exposure phases for mortality, behavioural changes and general state of colonies, including queen, brood and eggs. Weather data were collected daily.
Data analysis:	Not stated, not needed.

Results:

During Trials 1 and 2, exposure to dimethoate caused 6.3 and 10.5-fold increase, respectively, in bee mortality relative to control hives. In addition, foraging, flight activity and egg production was severely disrupted following exposure to the toxic reference dimethoate. Exposure to A-7402 A did not have any effect on bee mortality relative to control hives and did not cause any significant observable changes in foraging activity or brood behaviour. Bee mortality data is presented in the table below.

Table B.9.4.2-1: Effect of A-7402 A on bee mortality during acclimatization and exposure phases

Treatment	Number of dead bees per day			
	Trial 1		Trial 2	
	Acclimatization	Exposure	Acclimatization	Exposure
Control	29	6	22	4
A-7402 A	37	6	19	6
Dimethoate	26	38	14	42

Exposure to spray applications of A-7402 A, at 2 L formulation/ha, did not have any significant effects on bee mortality, foraging behaviour, flight activity or the general state of queen, brood and eggs.

RMS comments:

The study was well performed and reported and is considered valid for the risk assessment.

Reference:	**Leopold, M.A. (1993). Toxicity study in foraging honey bees (*Apis mellifera* L.) under semi-field (tent) conditions with CDG 96430F. RCC Notox B.V., s'Hertogenbosch, The Netherlands. Unpublished report no. 286931. (Syngenta No. CGA 169374/1273). Study dates: 15 July -7 August 1992.**
Guideline:	BBA VI, 23-1.
GLP:	Yes.
Material and methods:	
Test substance:	CGD 96430F (equivalent to A-7402 A) containing 250 g/L difenoconazole, batch number P.111003.
Species:	Honey bees (*Apis mellifera*).
Treatments:	Two successive tests; On each occasion beehives, containing three combs, were placed in a plot of flowering *Phacelia* and each was covered with a gauze tent. After 8 days (Experiment 1) or 4 days (Experiment 2), spray solutions of A-7402 A, prepared in tap water, were sprayed over the crop and foraging bees at a rate of 200 L/ha to give an exposure concentration of 2 L A-7402 A /ha (or 500 g as/ha). The second experiment was initiated eighteen days after completion of the first test.
Number of replicates:	Each test incorporated one tent for the A-7402 A treatment, one tent for the control, in which crop and bees were sprayed with tap water, and one tent of a toxic standard in which crop and bees were sprayed with parathion (250 g as/L).
Duration:	4 or 8 days for acclimation, observations 4 days after treatment.
Test conditions:	Weather data was collected every tenth minutes in both experiments.
Observations:	Bees were monitored daily for up to 4 days for mortality, behavioural changes and

general state of colonies, including queen, brood and eggs.

Data analysis: Not stated, not needed.

Results:

In tents exposed to parathion, bee mortality was 6.9 to 17.7-fold greater than in control tents. In addition, foraging and flight activity were severely disrupted. In tents exposed to A-7402 A, bee mortality was 91-158% of that recorded in control tents. Flight density, foraging activity and brood health were not affected by exposure to A-7402 A. Bee mortality data is presented in the table below.

Table B.9.4.2-2: Effect of A-7402 A on bee mortality

Treatment	Total number of dead bees	
	Experiment 1	Experiment 2
Control	182	102
A-7402 A	167	162
parathion	1258	1807

RMS comments:

The study was well performed and reported. However, since the results regarding mortality differed significantly between the two experiments, it is difficult to draw any conclusions on possible treatment related effects from this study. Hence, the results do not add any useful information to the risk assessment.

B.9.4.3 Summary and risk assessment for honeybees

Descriptions of honey-bee acute oral and contact toxicity studies conducted with difenoconazole were provided in **Document M-II, Section 6**. In addition, acute oral studies were conducted with the formulation, A-7402 A, which is similar to A-7402 T. A summary of acute and contact oral toxicity endpoints from all studies is provided in the table below. For the use of difenoconazole for seed treatment in wheat, exposure is considered to be negligible.

Table B.9.4.3-1: Acute toxicity of difenoconazole to honey bees

Test substance	Endpoint	LD_{50} (µg as/bee)	Reference
Difenoconazole	48-h contact LC_{50}	>100	Hoxter and Jaber (1989)
	48-h contact LC_{50}	>100	Grieg- Smith (1990)
	48-h oral LC_{50}	>177	

In addition, two semi-field tests conducted under realisitic conditions were submitted by the notifier. The results indicated that no significant effects on bee mortality, foraging behaviour, flight activity or brood health are expected at the representative use of difenoconazole by spray application.

The available data fulfils the requirements of Annex II and III of 91/414 and are considered to be sufficient for the risk assessment for honey bees.

B.9.4.3.1 SEED TREATMENT WITH DIVIDEND 030FS

A risk assessment was provided by the notifier in **Document M-III, Section 6.** As difenoconazole is systemic, honey-bees may potentially be exposed by ingestion of nectar and pollen containing residues in crops grown from DIVIDEND 030FS treated seed. The maximum concentration of difenoconazole in nectar and pollen was assumed to be equivalent to the proposed seed-treatment rate, *i.e.*12.3 g as/ha. This assumption is very conservative, as degradation of difenoconazole in the soil or dilution due to uptake, translocation and metabolism within the crop plant, have not been taken into account. Furthermore, exposure is also likely to be limited as the proposed uses of DIVIDEND 030FS are on cereals, which are not particularly attractive to bees.

The ratio between maximum application rate and acute LD_{50} (Hazard Quotient) is used as an indication of potential risk (EPPO/CoE, 1993) and is calculated as follows:

$$\text{Hazard quotient} = \frac{\text{maximum application rate (g ai / ha)}}{\text{acute } LD_{50} \text{ (µg ai / bee)}}$$

The HQ for DIVIDEND 030FS was calculated by the notifier using an acute oral LD_{50} of 177 µg as/bee as shown in the table below. The resulting value is much lower than the trigger value of 50, indicating a negligible risk to bees.

Table B.9.4.3-2: Honey-bee hazard quotient for difenoconazole, calculated by the notifier.

Exposure route	LD_{50} (µg/bee)	Application rate (g as/ha)	Hazard quotient
Oral	177*	12.3**	0.069

**this value was changed by RMS, probably typing error in the notifier's assessment.*
***based on maximum seed treatment rate(60 mg as/kg seed) multiplied by the maximum seed planting rate (205 kg seed per hectare), RMS comment.*

RMS comments

There is no agreed standard for calculation of HQ for seed treatment products. However, the RMS agrees with the approach proposed by the notifier, and with the conclusion that DIVIDEND 030FS poses low risk to bees when used as a seed-treatment in wheat. No further refinement is needed.

B.9.4.3.2 SPRAY APPLICATION WITH SCORE 250EC

A risk assessment was provided by the notifier in **Document M-III, Section 6.** Applications of SCORE 250EC could result in exposure of honey-bees either by direct over-spray or by contact with residues on plants whilst bees are foraging for food. For this assessment, the maximum single application rate for each representative use pattern will be used to represent the worst-case scenario.

The potential risk of SCORE 250EC to honey-bees was estimated from a hazard quotient calculated as the ratio between the application rate and acute LD_{50}:

$$\text{Hazard quotient} = \frac{\text{maximum application rate (g ai/ha)}}{\text{acute } LD_{50} \text{ (µg ai/bee)}}$$

Hazard quotients for difenoconazole, calculated by the notifier using the highest single application rate of 125 g as/ha and the lowest acute toxicity endpoint for each exposure route, are presented in the table below.

Table B.9.4.3-3: Oral and contact exposure hazard quotient for bees exposed to difenoconazole by spray application.

Field rate (g as/ha)	Exposure route				Hazard quotient assessment trigger
	Oral		Contact		
	LD_{50} (µg as/bee)*	Hazard quotient	LD_{50} (µg as/bee)*	Hazard quotient	
Pome fruit 75 g as/ha	177	0.42	100	0.75	50
Carrots 125 g as/ha	177	0.71	100	1.25	50

**values corrected by the RMS since the originally proposed values were not considered to be valid.*

The hazard quotients for bees based on acute toxicity values are below the trigger value of 50. Furthermore, cage tests conducted with A-7402 A indicate that exposure to spray applications at 2 L formulation/ha (500 g as/ha), does not have any significant effects on bee mortality, foraging behaviour, flight activity or brood health.

RMS comments

The figures provided by the notifier were replaced by values assessed to be more valid by the RMS. This correction resulted in lower HQ values, but did not change the overall conclusion that the representative use of SCORE 250EC in pome fruit and carrots is of low risk to honey bees. No valid laboratory studies were available for the formulation, however, this data requirement is considered to be covered by the submitted semi-field studies. No further data is considered necessary.

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

The toxicity of difenoconazole to non-target arthropods was tested in laboratory, dose-response studies with the standard test species *Aphidius rhopalosiphi* and *Typhlodromus pyri*. These studies were mainly conducted with the difenoconazole formulation, SCORE 250EC (A-7402G), which is intended for application by foliar spray. In addition, studies were conducted with DIVIDEND 030FS (A-9142 G) on the ground-dwelling arthropods *Aleochara bilineata* and *Poecilus cupreus* as these were considered more likely to be exposed to treated seeds.

B.9.5.1 Laboratory tests

ACTIVE INGREDIENT

No studies were conducted with the active ingredient alone. However, the studies with the two sensitive standard species conducted with the formulated product Score 250EC are considered to fulfil the data requirements in Annex II of Directive 91/414, and to cover possible effects of the active ingredient.

Reference:	**Kleiner, R. (2000a). Acute dose-response toxicity of CGA 169374 EC (A-7402G) to the cereal aphid parasitoid *Aphidius rhopalosiphi* (Destefani-Perez) under laboratory conditions. Biochem Agrar, Cunnersdorf, Germany. Unpublished report number 99 10 48 083. Study dates 20 July – 5 October 1999 (Syngenta File No. CGA 169374/2095)**
Guideline:	IOBC (Mead-Briggs 1992, 1997).
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 G containing 265 g CGA 169374/L. Batch number P.706093.
Species:	Cereal aphid parasitoid *Aphidius rhopalosiphi*
Treatments:	Stock solutions of A-7402G, prepared in deionsied water, were sprayed onto glass plates at a rate of 200 L/ha to give nominal exposure concentrations of 18, 36, 72, 144 and 288 g as/ha. Plates were allowed to dry for 1 hour and assembled in an aluminium cage prior to the introduction of the wasps.
Number of animals:	Five female and five male wasps (<48 hours old) per replicate. The test incorporated 6 replicate cages for each exposure concentration, 6 replicates of a control treatment in which glass plates were sprayed with deionised water and 1 replicates of a toxic standard in which plates were sprayed with dimethoate (EC 400) at 0.85 mL/ha.
Duration:	48-hour exposure phase followed by a 24-hour parasitisation phase and 11-day incubation phase.
Test conditions:	Cages were maintained in a controlled environment chamber at 20±2°C with 69 – 97% relative humidity and continuous light of 1100 lux during the exposure and parasitisation phases, and a 16-hour day during mummy development. Food was provided as 1:2 solution of honey and water, as soaked cotton wool bung. *Rhopalosiphum padi* was used as host aphids.
Observations:	The condition of the wasps was assessed at 1, 24 and 48 hours after their introduction. For those treatments where 14 female wasps survived for 48 hours, each female wasp was transferred to an individual acrylic cylinder containing a pot of wheat infested with >100 nymphal aphids (II-III instar). After 24 hours the wasps were removed and the plants were maintained for a further 10-11 days for assessment of the number of aphid mummies.
Data analysis:	ANOVA for statistical significance. Corrected mortality and parasitation rate according to Abbot (1925). Dunnett's test for NOEC and LOEC, probit analysis according to Finney (1971).

Results:

Mortality and reproduction data are presented in Table B.9.5.1-1. Exposure to the toxic standard, dimethoate, caused 100% mortality of adult wasps within 24 hours. Exposure to A-7402G concentrations of 18 or 36 g as/ha did not have any significant effect on mortality. However, 48-hour corrected mortality was significantly increased to 26, 28 and 74% following exposure to 72, 144 and 288 g as/ha, respectively. The reproductive capability of surviving female wasps was not adversely affected by concentrations up to 72 g as/ha but the

number of aphid mummies was significantly reduced by 31 and 87% relative to control values following exposure to 144 and 288 g as/ha, respectively.

Table B.9.5.1-1: Effect of A-7402 G on mortality and reproduction in *Aphidius rhopalosiphi*

Nominal concentration (g as/ha)	Corrected mortality (%)		Number of aphid mummies 11 d after 24 h parasitisation period.	Parasitation efficiency (% relative to control)
	24 hours	48 hours		
0	0a	0a	12.9	100
18	0	4	13.2	102
36	12	14	14.2	110
72	17	26*	12.5	97
144	19	28*	8.9*	69*
288	31*	74*	1.7n.d.	13n.d.
Toxic standard	100*	n.a.	n.a.	n.a.

[a]control mortalities were 3.3% at 24 hours and 5% at 48 hours
**significantly different from control (p≤0.05)*
n.a. not applicable due to 100% mortality
n.d. significance not determined

Based on mortality data, the 48-hour LR_{50} for A-7402 G in *Aphidius rhopalosiphi* was estimated to be 178 g as/ha. In the absence of significant adverse effects on mortality or reproduction at doses up to 36 g as/ha, the NOEC was considered to be 36 g as/ha.

RMS comments:

The study was well performed and reported and is considered to be valid for the risk assessment.

Reference:	**Kleiner, R. (2001). Acute dose-response toxicity of CGA 169374 EC (A-7402G) to the predatory mite, *Typhlodromus pyri* (Scheuten), under laboratory conditions. Biochem Agrar, Cunnersdorf, Germany. Unpublished report number 99 10 48 084. Study dates 23 August – 30 September 1999 (Syngenta File No. CGA 169374/2131)**
Guideline:	IOBC (Overmeer, 1988); Louis and Ufer (1995).
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 G containing 265 g CGA 169374/L. Batch number P.706093.
Species:	Predatory mite *Typhlodromus pyri*
Treatments:	The study was conducted as a glass-plate mortality test followed by a fecundity test. Stock solutions of A-7402 G, prepared in deionised water, were sprayed onto glass plates at a rate of 200 L/ha to give nominal exposure concentrations of 18, 36, 72, 144 and 288 g as/ha. Plates were allowed to dry for 1 hour and assembled in an exposure cage prior to the introduction of the mites.
Number of animals:	The test incorporated five replicate cages for each exposure concentration, each with 20 protonymphs, three replicates of a control treatment in which glass plates were sprayed with deionised water, and three replicates of a toxic standard, in which plates were sprayed with dimethoate (EC 400) at 8 g as/ha.
Duration:	14 days exposure. 3 additional days for larval hatch.

Test conditions:	Cages were maintained in a controlled environment chamber at 25±2°C and 54 – 93% relative humidity, with a 16-hour day of 1100 lux. Pollen of pine and birch was provided as food.
Observations:	The numbers of surviving mites were recorded on days 1, 3, 7, 9, 11 and 14 days after application. For those treatments where sufficient females survived, the number of eggs laid were collected and counted on days 7, 9, 11 and 14. After transfer to hatching cages, the cumulative number of hatched eggs was recorded on days 9, 11, 14 and 17.
Data analysis:	ANOVA for statistical significance. Corrected mortality and parasitation rate according to Abbot (1925). Dunnett's test for NOEC and LOEC, probit analysis according to Finney (1971).

Results:

Mortality and reproduction data are presented in Table B.9.5.1-2. Exposure to dimethoate caused 100% mortality in predatory mites within 7 days. Exposure to A-7402 G concentrations of 18 g as/ha did not have any significant effect on the mortality or fecundity of predatory mites. However, after 14 days mortality was significantly increased to 13, 38, 94 and 100% at concentrations of 36, 72, 144 and 288 g as/ha, respectively. The number of eggs laid by surviving female mites was not adversely affected by concentrations up to 36 g as/ha but was significantly reduced by 41 and 100% of control values following treatment with 72 and 144 g as/ha, respectively. Similarly, hatching rates were not adversely affected by treatment with concentrations up to 36 g as/ha whereas exposure to 72 g as/ha significantly reduced the number of larvae produced per egg by 10% of control values.

Table B.9.5.1-2: Effect of A-7402 G on mortality and reproduction in *Typhlodromus pyri*

Nominal concentration (g as/ha)	Corrected mortality (%)			Reproduction			
	Day 1	Day 7	Day 14	Number of eggs	Eggs per female	Number of larvae	Larvae per egg
0	0	0	0	402	7.68	352	0.88
18	0	-1	5	415	7.30	352	0.85
36	0	4	13*	335	7.37	287	0.86
72	1	13	38*	178	4.56*	142	0.80*
144	3	65*	94*	0	0*	n.a.	n.a.
288	14	100*	100*	n.a.	n.a.	n.a.	n.a.
Toxic standard	69*	100*	n.a.	n.a.	n.a.	n.a.	n.a.

Control mortalities were 0% on day 1, 1% on day 7 and 5% on day 14
**significantly different from control (p≤0.05)*
n.a. not applicable due to high mortality

Based on mortality data, the 7-day LR_{50} for A-7402 G in *Typhlodromus pyri* was estimated to be 111.6 g as/ha (CI, p≤0.05=76-165.6 g as/ha). The 14-day NOEC was considered to be 18 g as/ha.

RMS comments:

The study was well performed and reported and is considered to be valid for the risk assessment. Based on mortality data from day 14, the LR_{50} value would have been slightly lower. However, using the data from day 7

is consistent with the recommendations in the referred guidelines and therefore no further calculations are needed.

PLANT PROTECTION PRODUCT

DIVIDEND 030FS

Reference:	**Grimm, C. (1999a). Acute toxicity of CGA 169374 FS 30 (A-9142 G) to the rove beetle *Aleochara bilineata* Gyll. (Coleoptera, Staphylinidae) Novartis Crop Protection AG, Basel, Switzerland. Unpublished report no. 982924. Study dates 28 June–12 July 1999 (Syngenta File No. CGA 169374/1959)**
Guideline:	IOBC (Samsoe-Petersen, 1992).
GLP:	Yes.
Material and methods:	
Test substance:	A-9142 G containing 30.6 g/L CGA 169374. Batch number P.902001.
Species:	Rove beetle, *Aleochara bilineata*
Treatments:	Beetles were exposed to wheat seeds, treated with A-9142 G at a rate of 2 mL formulation/kg seed. Two seeds were placed in each exposure chamber resulting in an estimated seed density of 379 kg seed/ha (compared to the target density 300 kg seeds/ha).
Number of animals:	One adult female and 100 *Protophormia* eggs were then introduced into each chamber. The test incorporated ten replicate chambers for the test substance treatment, ten replicates for an untreated control, and ten replicates for the toxic standard in which seeds were treated with methyl parathion (WP 40) at 4 g formulation/kg seed.
Duration:	96 hours exposure phase, 10 days reproductive phase.
Test conditions:	Chambers were maintained in a controlled environment at 20±2°C and 66 – 91% relative humidity, with a 16 hour day of <1000 lux. Each chamber contained a layer of pure quartz sand (18 g moistened sand, with 1 mL of water per 30 g sand) as substrate.
Observations:	Beetles were assessed for mortalities and supplied with fresh *Protophormia* eggs daily. After 4 days, *Aleochara* eggs were collected from exposure chambers and transferred to hatching chambers. After a further 10 days, the number of hatched larvae was recorded.
Data analysis:	Fisher exact test, Shapiro and Wilk test, Mann-Whitney U-test. Abbot (1925) for corrected mortality.

Results:

Mortality, food consumption and fecundity data are presented in Table B.9.5.1-3. Exposure to the toxic standard caused 100% mortality of adult beetles within 4 days. Exposure to A-9142 G for 4 days did not have any significant effect on the mortality, food consumption or fecundity.

Table B.9.5.1-3: Effect of A-9142 G on mortality, food consumption and fecundity in *Aleochara bilineata*

Treatment	Mortality (%) on day 4	Food consumption	Eggs per female	Viable eggs per female
Control	10 (0)	224	40	35.1
A-9142 G	30 (corrected 22.2)	272	40.7	36.7
Toxic standard	100*	n.a.	n.a.	n.a.

**significantly different from control ($p \leq 0.05$)*
n.a. not applicable

Exposure to wheat seeds treated with A-9142 G at 2 mL formulation/kg seed (61.2 mg as/kg seed) and seed density of 379 kg/ha (equivalent to 23.2 g as/ha), did not have a significant effect on mortality or fecundity of the rove beetle, *Aleochara bilineata*.

RMS comments:

The study was well performed and reported and is considered to be valid for the risk assessment. The hectare dose, 23 g as/ha, was almost two times the representative GAP for seed treatment (12.3 g as/ha). Hence, the exposure levels in this study can be regarded as a worst case.

Reference:	**Grimm, C. (1999b). Acute toxicity of CGA 169374 FS 30 (A-9142 G) to the predatory ground beetle *Poecilus cupreus* L. (Coleoptera: Carabidae). Novartis Crop Protection AG, Basel, Switzerland. Unpublished report no. 982923. Study dates 25 June –12 July 1999 (Syngenta File No. CGA 169374/1960)**
Guideline:	IOBC (Heimbach, 1992).
GLP:	Yes.
Material and methods:	
Test substance:	A-9142 G containing 30.6 g/L CGA 169374. Batch number P.902001.
Species:	Ground beetle *Poecilus cupreus*
Treatments:	Adult beetles were transferred to exposure chambers and exposed to wheat seeds, treated with A-9142 G at a rate of 2 mL formulation/kg seed. Fifteen seeds were placed in each chamber to give an estimated seed density of 307 kg seed/ha.
Number of animals:	Three males and three females per exposure chamber. The test incorporated five replicate chambers for the test substance treatment, five replicates for an untreated control in which seeds were treated with deionised water, and five replicates for the toxic standard in which seeds were treated with methyl parathion (WP 40) at 4 g formulation/kg seed.
Duration:	14 days exposure.
Test conditions:	Chambers were maintained in a controlled environment at 20±2°C and 66 - 91% relative humidity, with a 16 hour day of 500-1500 lux. Beetles were fed with one *Calliphora* sp. fly pupa per beetle immediately prior to exposure and on test days 2, 4, 7 and 10. Each chamber contained 250 g dw of pure quartz sand, moistened with 46 mL of water to reach 70% of the maximum water holding capacity.
Observations:	Beetles were inspected for mortalities and symptoms of toxicity at 2, 4, 6 and 24 hours and 2, 4, 7, 10 and 14 days after application. The number of fly pupae consumed was also recorded on days 2, 4, 7, 10 and 14 days.

Data analysis: Fisher exact test for mortality, Dunnett's and Mann-Whitney U-test for significance. Corrected mortality according to Abbott (1925).

Results:

Mortality and food consumption data are presented in Table B.9.5.1-4. Exposure to the toxic standard caused 100% mortality within 2 days of treatment. Neither control beetles nor those exposed to A-9142 G suffered any mortalities during the 14-day exposure phase. Beetles did not exhibit any abnormal behaviour and food consumption was not significantly affected by exposure to A-9142 G.

Table B.9.5.1-4: Effect of A-9142 G on mortality and food consumption in *Poecilus cupreus*.

Treatment	Mortality (%) Day 14	Mean fly pupa consumption per beetle
Control	0	0.06
A-9142 G	0	0.06
Toxic standard	100*	n.a.

**significantly different from control ($p \leq 0.05$)*
n.a. not applicable

Exposure to wheat seeds treated with 2 mL A-9142 G/kg seed and a seed density of 307 kg/ha, (equivalent to 18.8 g as/ha) had no significant effect on mortality or behaviour of the ground beetle, *Poecilus cupreus*.

RMS comments:

The study was well performed and reported and is considered valid for the risk assessment. The hectare dose 18 g as/ha can be compared with the representative GAP for seed treatment, 12.3 g as/ha. Hence, the exposure levels in this study can be regarded as a worst case.

Reference: **Reber, B. (1999). Acute toxicity of CGA 169374 FS 30 (A-9142 G) to larvae of the predatory ground beetle *Poecilus cupreus* L. (Coleoptera: Carabidae). Novartis Crop Protection AG, Basel, Switzerland. Unpublished report no. 991501. Study dates 7 July - 9 September. 1999 (Syngenta File No. CGA 169374/1975)**

Guideline: IOBC (Heimbach, 1998).

GLP: Yes.

Material and methods:

Test substance: A-9142 G containing 30.6 g/L CGA 169374. Batch number P.902001.

Species: Ground beetle *Poecilus cupreus.*

Treatments: Beetle larvae, 12-48 hours old, were transferred to exposure chambers and exposed to wheat seeds, treated with A-9142 G at a rate of 2 mL formulation/kg seed. One seed was placed in each chamber to give an estimated seed density of 943 kg seed/ha.

Number of animals: The test incorporated 40 replicate chambers of one larva for the test substance treatment, 40 replicates of an untreated control in which seeds were treated with deionised water, and 40 replicates of the toxic standard in which seeds were treated with methyl parathion (WP 40) at 4 g formulation/kg seed.

Duration:	48-51 days exposure (up to hatching of the adult beetles).
Test conditions:	Chambers were maintained under a controlled environment at 20±2°C and 65 – 84% relative humidity, with constant darkness. Each chamber (35 mL glass tubes) contained 28 g moist LUFA 2.1 soil (>80% sand, 30% of maximum water holding capacity of 30%). Adult beetles were fed with cut beetle larvae of *Tenebrio molitor*. Larvae were fed with one half of a *Calliphora* sp. fly pupa each, immediately after exposure and three times a week, for the first two weeks of the study. Thereafter, feeding was reduced to twice a week and subsequently to once a week when larvae reached the pupal stage.
Observations:	Larvae were inspected for mortalities at each feeding date. The date of the appearance of the pupal stage and hatched adult were also recorded. Beetles were weighed when their skin became black.
Data analysis:	Fisher exact test for mortality, Dunnett's and Mann-Whitney U-test for significance. Corrected mortality according to Abbott (1925).

Results:

Mortality, development time, body weight and sex ratio data are presented in Table B.9.5.1-5. Exposure to the toxic standard caused 100% mortality within 6 days. Exposure to A-9142 G did not have a significant effect on mortality, development time, adult body weight or sex ratio.

Table B.9.5.1-5: Effect of A-9142 G on mortality and food consumption in *Poecilus cupreus*.

Treatment	Mortality (%) at end of exposure phase	Development time (larva-adult; days)	Adult body weight (mg)	Sex ratio (% female: male)
Control	27.5	49.3	52.6	55.2:44.8
A-9142 G	27.5	49.3	53.8	48.3:51.7
Toxic standard	100*	n.a.	n.a.	n.a.

**significantly different from control ($p \leq 0.05$)*
n.a. not applicable

Exposure of *Poecilus cupreus* larvae to wheat seeds treated with A-9142 G at 2 mL formulation/kg seed and a seed density of 937 kg/ha (equivalent to 56.4 g as/ha), had no significant effects on mortality, development time, adult body weight or sex ratio.

RMS comments:

Although it was noted that the mortality in the control was somewhat high, the study was well performed and reported and is considered as valid for the risk assessment. The hectare dose 56.4 g as/ha can be compared with the representative GAP for seed treatment, 12.3 g as/ha. Hence, the exposure levels in this study can be regarded as a worst case.

SCORE 250EC

Reference:	**Nienstedt, K.M. (1999a). CGA 169374 EC 250 (A-7402 G): Laboratory acute toxicity test with the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera: Aphidiidae). Springborn Laboratories (Europe), Horn, Switzerland. Unpublished report number 98-226-1047. (Syngenta No. CGA 169374/1963). 15-28 September 1998.**
Guideline:	IOBC (Polgar, 1988; Mead-Briggs 1992, 1997).
GLP:	Yes.

Material and methods:

Test substance:	A-7402 G containing 253 g CGA 169374/L. Batch number P.612076.
Species:	Cereal aphid parasitoid *Aphidius rhopalosiphi*.
Treatments:	Stock solutions of A-7402 G, prepared in deionised water, were sprayed onto glass plates at a rate of 200 L/ha to give nominal exposure concentrations of 5.06, 126.5 and 253 g as/ha. Plates were allowed to dry for 1 hour and assembled in an aluminium cage prior to the introduction of the wasps (<48 hours old). For those treatments where 14 female wasps survived for 48 hours, each female wasp was transferred to an individual acrylic cylinder containing a pot with 5-10 barley plants (5 – 15 cm tall) infested with 40 nymphal aphids (II-III instar) and 40 aphids at other development stages. After 24 hours the wasps were removed and the number of live females was recorded. The plants were maintained for a further 10 days for assessment of the number of aphid mummies.
Number of animals:	The test incorporated 4 replicate cages for each exposure concentration, 4 replicates of a control treatment in which glass plates were sprayed with deionised water, and 4 replicates of a toxic standard (each with 10 wasps per replicate) in which plates were sprayed with dimethoate (Perfekthion) at 0.17 mL/ha. For the fecundity phase, 14 replicates per treatment, with 1 wasp per replicate were used.
Duration:	48 hours exposure phase followed by a 24 hours parasitisation phase and 10 days incubation phase.
Test conditions:	Cages were maintained in a controlled environment chamber at 18-21°C and 75 – 93% relative humidity, with a 16-hour day of 1000 lux during the exposure and parasitisation phases, and >2000 lux during mummy development.
Observations:	The condition (“alive and healthy”, “affected”, “moribund” or “dead”, respectively) of the wasps was assessed at 1, 24 and 48 hours after their introduction. The number of aphid mummies was assessed after a further 10 days.
Data analysis:	Fisher exact test for mortality, Shapiro-Wilk’s test for fecundity. ANOVA. Beneficial capacity calculated according to Overmeer and Van Zon (1982).

Results:

Mortality and reproduction data are presented in Table B.9.5.1-6. Exposure to the toxic standard, dimethoate, caused 100% mortality of adult wasps within 24 hours. Control wasps or those exposed to A-7402 G concentrations of 5.06 or 126.5 g as/ha did not suffer mortalities or show abnormal behaviour during the 48-hour exposure phase. However, of those exposed to 253 g as/ha, 47.5% died and 15% exhibited uncoordinated

behaviour. Similarly, female control wasps or those exposed to A-7402 G concentrations of 5.06 or 126.5 g as/ha did not suffer any mortalities during the first 24 hours of the fecundity test whereas 57.1% of those exposed to 253 g as/ha died. The number of aphid mummies produced by each surviving female was not significantly affected by 5.06 or 126.5 g as/ha but was significantly reduced from 22.3 in the control treatment to 9.5 in the 253 g as/ha treatment. The resulting E-values (representing "total effect") for beneficial capacity were calculated to 4, 27 and 57% for the lowest, medium and high treatment rates, respectively.

Table B.9.5.1-6: Effect of A-7402 G on mortality and reproduction in *Aphidius rhopalosiphi*

Nominal concentration (g as/ha)	Mortality (%)		Fecundity test		
	24 hours	48 hours	Female mortality	Number of aphid mummies per female	% reduction in number of aphid mummies
0	0	0	0	22.3±10.3	-
5.06	0	0	0	21.4±11.7	4.0%
126.5	0	0	0	16.2±4.9	27%
253	0	47.5±33.0*	57.1±51.4*	9.5±8.4*	57%
Toxic standard	100	n.a.	n.a.	n.a.	n.a.

**significantly different from control (p≤0.05)*
n.a. not applicable due to 100% mortality

According to the author of the study, exposure to fresh A-7402 G residues at concentrations up to and including 126.5 g as/ha did not have a significant effect on the mortality or reproduction of *Aphidius rhopalosiphi.* Exposure to A-7402 G residues of 253 g as/ha caused significant increases in mortality and reduction in reproductive capacity. Therefore, the NOEC was considered to be 126.5 g as/ha.

RMS comments:

The study was well performed and reported and is considered as valid for the risk assessment. The large variability in reproduction data for both control and treated group reduces the statistical power of the analysis. Therefore, for precautionary reasons, the effects at the mid dose could also be considered as significant, and the NOEC can be set to 5.06 g as/ha. However, since the risk assessment is based on the ER_{50} value, this will have no impact on the risk assessment.

Reference:	**Nienstedt, K.M. (1999b). CGA 169374 EC 250 (A-7402 G): Laboratory acute toxicity test with the predacious mite *Typhlodromus pyri* (Acari: Phytoseiidae). Springborn Laboratories (Europe), Horn, Switzerland. Unpublished report number 98-231-1047. (Syngenta No. CGA 169374/1978). 28 July – 11 August 1998.**
Guideline:	IOBC (Overmeer, 1988; Louis and Ufer, 1995).
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 G containing 253 g CGA 169374/L. Batch number P.612076.
Species:	Predacious mite *Typhlodromus pyri*.
Treatments:	Stock solutions of A-7402 G, prepared in deionised water, were sprayed onto glass plates at a rate of 200 L/ha to give nominal exposure concentrations of 5.06, 126.5 and 253 g as/ha. Plates were allowed to dry for 1 hour and assembled in an exposure

chamber prior to the introduction of the predatory mites (1-day old protonymphs).

Number of animals: The test incorporated five replicate chambers for each exposure concentration, five replicate controls in which glass plates were sprayed with deionised water, and five replicates of a toxic standard (each with 20 protonymphs) in which plates were sprayed with ethyl parathion (500 g/L EC) at 0.036%.

Duration: 14 days.

Test conditions: Cages were maintained in a controlled environment chamber at 25±2°C and relative humidity of 65 – 95%, with a 16 hour day of 1000-1500 lux. Test animals were fed with walnut and apple pollen, and had continuous supply with clean tap water.

Observations: Numbers of surviving mites were recorded 3, 7, 10 and 14 days after application. The gender of surviving mites was determined from day 7 onwards and the numbers of eggs and hatched larvae were recorded on days 7, 10 and 14.

Data analysis: Yates corrected Chi-square test for mortality, Shapiro-Wilk's test for fecundity. ANOVA and Tukey HSD test. Corrected mortality according to Abbot (1925), reproduction according to Overmeer (1988).

Results:

Mortality and reproduction data are presented in Table B.9.5.1-8. Exposure to ethyl parathion caused 71% mortality by day 14 and a significant 67% reduction in the number of eggs per female. Exposure to A-7402 G at 5.06 g as/ha did not have any significant effect on the mortality or fecundity of predatory mites. However, for those mites exposed to 126.5 and 253 g as/ha, mortality was significantly increased to 30 and 71%, respectively, by day 7, 40 and 83%, respectively, by day 10 and 47 and 91%, respectively, by day 14. Similarly, the number of eggs produced per female was significantly reduced from 6.54 for control mites to 4.09 and 1.5 for those exposed to 126.5 and 253 g as/ha, respectively.

Table B.9.5.1-8: Effect of A-7402 G on mortality and reproduction in *Typhlodromus pyri*

Nominal concentration (g as/ha)	Mortality (%)				Fecundity	
	Day 3	Day 7 [a]	Day 10	Day 14	Eggs per female	Eggs/female/day
0	1	3 (0)	5	8	6.54	0.93
5.06	1	3 (0)	9	12	5.10	0.73
126.5	22	30* (28)	40*	47*	4.09*	0.58
253	53	71* (70)	83*	91*	1.50*	0.21
Toxic standard	42	56* (55)	62*	71*	2.13	0.30

[a] *corrected mortality shown in brackets*
**significantly different from control (p≤0.05)*

Based on mortality data, the 7-day LR_{50} for A-7402 G in *Typhlodromus pyri* was estimated to be >126.5 g as/ha. In the absence of significant adverse effects on mortality or fecundity at 5.06 g as/ha, the 14-day NOEC was considered to be 5.06 g as/ha.

RMS comments:

The study was well performed and reported and is considered as valid for the risk assessment. LR_{50} was calculated for the first 7 days after treatment, although observations were continued for 14 days. This is in line with the referred test guidelines.

Reference:	**Kleiner, R. (2000b). The effects of A-7402 G (Bardos Neu, 250 g/L difenoconazole) on the green lacewing *Chrysoperla carnea* STEPH. Under laboratory conditions. Biochem Agrar, Cunnersdorf, Germany. Unpublished report number 99 10 48 055. (Syngenta No. CGA 169374/2040). 6 September - 26 October 1999.**
Guideline:	IOBC (Bigler, 1988). Ring test method (Vogt *et al* 1997); OECD proposal (Vogt *et al* 1999).
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 G containing 253 g CGA 169374/L. Batch number P.612076.
Species:	Green lacewing *Chrysoperla carnea* STEPH.
Treatments:	Stock solutions of A-7402 G, prepared in deionised water, were sprayed onto glass plates at a rate of 200 L/ha to give nominal exposure concentrations of 4.05, 101.2 and 202.4 g as/ha. After drying for 1 hour, 10 glass rings (height 4 cm) were placed on each plate and one first instar larva was introduced into each ring. After pupation the glass rings were closed with gauze to contain the hatching lacewings. Adults were collected over a 7-day period from first hatching and separated into groups of 5 females and 3 males, prior to being transferred to oviposition cages for the reproduction phase of the test. One week after the first egg laying, the gauze (on which eggs were deposited) on each cage was changed every 24 hours for two weeks.
Number of animals:	The mortality test incorporated 5 replicate plates, each with 10 larvae for each exposure concentration, five replicates of the control treatment in which glass plates were sprayed with deionised water, and five replicates of a toxic standard in which plates were sprayed with dimethoate (as an EC 400 formulation) at 45 mL/ha. The reproduction test inluded 5 females and 3 males per replicate, and at least 3 replicates per treatment group.
Duration:	Mortality test 27 days, fecundity test 22 days.
Test conditions:	Test chambers were maintained in a controlled environment chamber at 20-29°C and a relative humidity of 56 – 87%, with a 16-hour day of 2000 lux.
Observations:	The numbers of surviving larvae, pupae and adults were recorded daily for 27 days. Eggs laid on the gauze were collected twice weekly, such that each sample collected comprised eggs laid over a 24-hour period. After counting, eggs were transfered to a hatching cage and the number of hatched larvae was recorded daily for 6 days.
Data analysis:	Dunnett's test. Corrected mortality and reproduction according to Abbot (1925) and Schneider-Orelli (1947).

Results:

Mortality and reproduction data are presented in Table B.9.5.1-9. Exposure of lacewing larvae to the toxic standard caused 90% mortality by day 27. Exposure to A-7402 G concentrations of 4.05 and 101.2 g as/ha did not have any significant effect on the mortality of adult lacewings or their capacity for the production of eggs, viable larvae and healthy adults.

For those larvae exposed to A7402G residues of 202.4 g as/ha, mortality was significantly increased from 8% in untreated larvae to 26%, while the number of eggs laid per female per day was significantly reduced from 16.6 in untreated larvae to 11.3 in exposed larvae. Furthermore, the number of eggs that successfully hatched was also significantly reduced from 83.4% in control females to 81.2% in those exposed to 202.4 g as/ha. When expressed as number of viable eggs per female per day, lacewings exposed to residues of 202.4 g as/ha produced 33% fewer eggs than untreated lacewings.

Table B.9.5.1-9: Effect of A-7402 G on mortality and reproduction in *Chrysoperla carnea*

Nominal concentration (g as/ha)	Mortality of hatched adults (%) Day 27[a]	Reproduction		
		Number of eggs per female per days	Egg hatch success (%)	Number viable eggs per female per day
0	8 (0)	16.6	83.4	13.8
4.05	12 (4)	15.7	81.9	12.9
101.2	16 (9)	13.4	81.8	11.0
202.4	26* (20)	11.3*	81.2*	9.2*
Toxic standard	90 (89)	n.a.	n.a.	n.a.

[a]*corrected mortality shown in brackets*
**significantly different from control ($p \leq 0.05$)*
n.a. not applicable

Exposure to fresh A-7402 G residues at concentrations up to and including 101.2 g as/ha did not have a significant effect on the mortality or reproduction of *Chrysoperla carnea.* Exposure to A-7402 G residues of 202.4 g as/ha caused significant increases in mortality and reduction in reproductive capacity. Therefore, the NOEC was considered to be 101.2 g as/ha.

RMS comments:

The study was well performed and reported and is considered as valid for the risk assessment. The ER_{50} can be set to >202 g as/ha.

Reference:	**Kleiner, R. (1999). The effects of A-7402 G (Bardos Neu, 250 g/L difenoconazole) on the spider *Pardosa* spp.under laboratory conditions. Biochem Agrar, Cunnersdorf, Germany. Unpublished report number 99 10 48 054. (Syngenta No. CGA 169374/2041). 31 May – 14 June 1999.**
Guideline:	IOBC (Wehling et al 1998).
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 G containing 253 g CGA 169374/L. Batch number P.612076.
Species:	Lycosid wolf spider, *Pardosa* spp.
Treatments:	Adult spiders were collected from field populations were acclimatized to controlled environment for 4 days. 10 females and 10 males were transferred to plastic cages

	containing moist sand (ca 70% of maximum water holding capacity) which after a further 3 days, were treated with A-7402 G by spray application. Immediately prior to treatment, spiders were fed with two frozen onion flies each. Stock solutions of A-7402 G, prepared in deionised water, were sprayed into each cage at a rate of 400 L/ha to give nominal exposure concentrations of 4.05, 101.2 and 202.4 g as/ha
Number of animals:	The test incorporated one replicate cage for each exposure concentration, one replicate control, which was sprayed with deionised water, and one replicate of a toxic standard, which was sprayed with endosulfan (Thiodan 35 EC) at 30 g as/ha.
Duration:	14 days exposure.
Test conditions:	Temperature 19-22°C, relative humidity 70 - 87%, and a 16-hour day of 1120 Lux.
Observations:	Spiders were inspected for mortalities and symptoms of toxicity at 2, 4 and 6 hours and 4, 7, 9, 11 and 14 days after application. On each occasion, spiders were given onion flies such that each surviving spider was offered 11 flies over the test period. The number of flies consumed was recorded on days 1, 2, 3, 4, 7, 9, 11 and 14 days.
Data analysis:	Corrected mortality according to Schneider-Orelli.

Results:

Mortality and food consumption data are presented in Table B.9.5.1-9. Exposure to the toxic standard caused 100% mortality after 4 days. In contrast, exposure to A-7402 G concentrations up to 202.4 g as/ha did not have a significant effect on spider mortality or food consumption. The spiders did not exhibit any abnormal behaviour during the test.

Table B.9.5.1-9: Effect of A-7402 G on mortality and food consumption in *Pardosa spp.*

Nominal concentration (L formulation/ha)	Mortality (%) Day 14	Total number of flies consumed
0	0	156
4.05	0	174
101.2	10	171
202.4	5	173
Toxic standard	100	2

Direct over-spray with A-7402 G at concentrations up to and including 202.4 g as/ha, did not have a significant effect on mortality of *Pardosa* species.

RMS comments:

Only one replicate was used, resulting in low statistical power of the results. However, due to the low effect levels, there is a large margin to severe effects. The study was well performed and reported, and is considered as valid for the risk assessment.

Reference:	**Reber, B. (1999b). Acute toxicity of CGA 169374 EC 250 (A-7402 G) to the predatory ground beetle *Poecilus cupreus* L. (Coleoptera: Carabidae). Novartis Crop Protection AG, Basel, Switzerland. Unpublished report number 993512. (Syngenta No. CGA 169374/1964). 3 – 20 April 1999.**

Guideline:	IOBC (Heimbach, 1992).
GLP:	Yes.

Material and methods:

Test substance:	A-7402 G containing 253 g CGA 169374/L. Batch number P.612076.
Species:	Predatory ground beetle *Poecilus cupreus* L.
Treatments:	Adult beetles (2-3 weeks old) were acclimatized to controlled environment conditions. After 3 days, 3 females and 3 males were transferred to plastic cages containing moist sand which after a further 3 days were treated with A-7402 G by spray application. Stock solutions of A-7402 G, prepared in deionised water, were then sprayed into each cage at a rate of 200 L/ha to give nominal exposure concentrations of 6.072, 30.36, 151.8 and 303.6 g as/ha. As toxic standard, pyrazophos (Afgugan EC30) was applied at 30 g as/ha.
Number of animals:	The test incorporated five replicate cages for each exposure concentration, five replicate control cages that were sprayed with deionised water, and five replicates of the toxic standard.
Duration:	14 days exposure.
Test conditions:	Temperature 20±2°C, relative humidity 75±15%, with a 16-hour day of 500-1500 Lux. Beetles were fed with one *Calliphora* sp. fly pupa per beetle immediately prior to exposure and on test days 2, 4, 7 and 10.
Observations:	Beetles were inspected for mortalities and symptoms of toxicity at 2, 4, 6 and 24 hours and 2, 4, 7, 10 and 14 days after application. The number of fly pupa consumed was also recorded on days 2, 4, 7, 10 and 14 days.
Data analysis:	Fisher exact test, Mann-Whitney U-test. Corrected mortality according to Abbot (1925).

Results:

Mortality and food consumption data are presented in Table B.9.5.1-10. Exposure to the toxic standard caused 100% mortality within 2 days of treatment. The control beetles or those exposed to A-7402 G did not suffer any mortalities during the 14-day exposure phase. The surviving beetles did not exhibit any abnormal behaviour and food consumption was not significantly affected by exposure to A-7402 G.

Table B.9.5.1-10: Effect of A-7402 G on mortality and food consumption in *Poecilus cupreus*

Nominal concentration (g as/ha)	Mortality (%) Day 14	Mean fly pupa consumption per beetle
0	0	0.15
6.072	0	0.16
30.36	0	0.16
151.8	0	0.16
303.6	0	0.16
Toxic standard	100	n.a.

n.a. not applicable

Direct over-spray with A-7402 G at concentrations up to and including 303.6 g as/ha, did not have a significant effect on mortality of the ground beetle, *Poecilus cupreus.*

RMS comments:

The study was well performed and reported and is considered as valid for the risk assessment.

B.9.5.2 Extended laboratory studies

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Reference:	**Grimm, C. (1999). Toxicity of CGA 169374 EC 250 (A-7402 G) to the predacious mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) under extended laboratory conditions. Novartis Crop protection AG, Basel, Switzerland. Unpublished report number 983929. (Syngenta No. CGA 169374/1981). 14 September - 19 October 1999.**
Guideline:	IOBC (Overmeer, 1988; Oomen, 1988).
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 G containing 253 g CGA 169374/E. Batch number P.612076.
Species:	Predatory mite *Typhlodromus pyri* Scheuten.
Treatments:	Leaf discs were placed inside petri dishes and sprayed with stock solutions of A-7402 G, prepared in deionised water, at a rate of 200 L/ha to give nominal exposure concentrations of 6.072, 30.36, 151.8 and 303.6 g as/ha. After air-drying, 10 mites (1-day old protonymphs) were introduced into each petri dish.
Number of replicates:	The test incorporated ten replicate dishes for each exposure concentration, ten replicates of the control treatment, which was sprayed with deionised water, and ten replicates of a toxic standard, in which plates (each with 10 protonymph mites, 1-day old) were sprayed with dimethoate (as Perfekthion EC 400) at 25 mL/ha.
Duration:	14 days.
Test conditions:	Dishes were maintained in a controlled environment chamber at 25±2°C and a relative humidity of 75 – 79%, with a 16 hour day of 2000 lux.
Observations:	Numbers of surviving mites were recorded 3, 7, 10 and 14 days after application. The gender of surviving mites was determined from day 7 onwards and the number of eggs and hatched larvae were recorded on days 7, 10 and 14.
Data analysis:	Fisher exact test, Bartlett test, Dunnett's test, Mann-whitney U-test. Corrected mortality according to Abbot (1925).

Results:

Mortality and reproduction data are presented in Table B.9.5.2-1. Exposure to the toxic standard caused 100% mortality within 7 days. Exposure to A-7402 G at 6.072 or 30.36 g as/ha did not have any significant effect on the mortality of predatory mites. However, for those mites exposed to 151.8 and 303.6 g as/ha, mortality was significantly increased to 19 and 39%, respectively, by day 3, to 42 and 67%, respectively, by day 7, to 52 and 75%, respectively, by day 10 and to 55 and 80%, respectively, by day 14. The number of eggs produced per

female per day was not significantly affected by 6.072, 30.36 or 151.8 g as/ha but was significantly reduced from 2.44 for control mites to 0 for those exposed to 303.6 g as/ha.

Table B.9.5.2-1: Effect of A-7402 G on mortality and reproduction in *Typhlodromus pyri*

Nominal concentration (g as/ha)	Mortality (%)				Fecundity
	Day 3	Day 7[a]	Day 10	Day 14	Eggs/female/day
0	4	10 (0)	16	23	2.44
6.072	0	6 (-4.4)	12	17	1.97
30.36	3	13 (3.3)	23	26	2.34
151.8	19*	42* (35.6)	52*	55*	1.86
303.6	39*	67*(63.3)	75*	80*	0*
Toxic standard	99*	100* (100)	n.a.	n.a.	n.a.

[a] *corrected mortality shown in brackets*
**significantly different from control ($p \leq 0.05$)*
n.a. not applicable

Exposure to fresh A-7402 G residues at concentrations up to and including 30.36 g as/ha did not have a significant effect on the mortality or fecundity of *Typhlodromus pyri.* Exposure to A-7402 G residues of 151.8 g as/ha caused significant increases in mortality but had no effect on the fecundity of surviving females. Exposure to residues of 303.6 g as/ha caused significant increases in mortality and reductions in fecundity. Therefore, the NOEC was considered to be 30.36 g as/ha.

RMS comments:

The study was well performed and reported and is considered as valid for the risk assessment. LR_{50} was calculated for the first 7 days after treatment, although observations were continued for 14 days. This is in line with the referred test guidelines.

Reference:	**Walker, H.M. (2001). Difenoconazole: A tier II laboratory study to determine the effects of a 250 g/L EC formulation (A-7402 G) on the green lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae). Ecotox Limited, Tavistock, Devon. (Syngenta No. CGA 169374/2205). 4 October – 3 December 2001.**
Guideline:	ESCORT (Barrett et al, 1994) Vogt et al, 2000.
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 G containing 253 g CGA 169374/L. Batch number P.612076.
Species:	Green lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae).
Treatments:	Stock solutions of A-7402 G, prepared in deionised water, were sprayed onto bean leaves at a rate of 200 L/ha to give nominal exposure concentrations of 14.45, 28.35, 75, 125, 202.5 and 287.5 g as/ha. After drying for one hour, a glass ring was placed over each leaf and secured to form an exposure chamber. One first instar larva (2-3 days old) was introduced into each ring. After 26 days, all pupae were transferred into culturing chambers and assessments of adult emergence were made 2 or 3 times per week until all had emerged or died. Surviving adults were transferred to clean chambers for the fecundity phase, which was initiated one week after the first egg laying in control cultures. Three egg production assessments were made over the following two weeks. For this purpose,

clean muslin was placed over each chamber and the numbers of eggs on the muslin and inside the chamber were recorded after 24 hours. The muslin was then transferred to plastic boxes for assessments of the number of emerging larvae.

Number of animals: The test incorporated 30 test chambers (in 3 replicate groups of 10) for each exposure concentration, the control treatment, in which leaves were sprayed with deionised water, and a toxic standard, in which leaves were sprayed with dimethoate (400 g/L) at 200 g as/ha.

Duration: Ca 50 days.

Test conditions: Test chambers were maintained in a controlled environment chamber at 25±2°C and relative humidity of 60 – 90%, with a 16-hour day.

Observations: Assessments of mortality and adult emergence were made twice a week until 26 days after treatment, by which time all larvae had pupated or died. Egg production monitored for two weeks thereafter.

Data analysis: Corrected mortality according to Abbot (1925). Fecundity data compared with historical control data according to Vogt et al. (2000). No statistical analysis of the data.

Results:

Mortality and reproduction data are presented in Table B.9.5.2-2. Exposure of lacewing larvae to the toxic standard caused 100% mortality. Exposure of lacewing larvae to A-7402 G concentrations of between 14.45 and 287.5 g as/ha caused juvenile mortality rates of 26.7 to 41.4% compared to a mortality rate of 20% in untreated larvae. Mortality was not correlated with exposure concentration.

Neither egg production nor hatching rate was correlated with A-7402 G concentration on any assessment occasion.

Table B.9.5.2-2: Effect of A-7402 G on mortality and reproduction in *Chrysoperla carnea*

Nominal concentration (g as/ha)	Larva and pupae mortality (%) Day 26[a]	Reproduction					
		Day 7		Day 12		Day 24	
		Eggs/female	Hatching success (%)	Eggs/female	Hatching success (%)	Eggs/female	Hatching success (%)
0	20 (0)	16.1	76.3	26.1	86.6	17.0	90.9
14.45	38.5 (23.1)	0	n.a.	20.3	87.5	30.7	100
28.35	41.4 (26.8)	7.6	76.5	21.2	100	31.8	100
75	26.7 (8.4)	23.6	73.6	26.0	100	17.7	88.6
125	33.3 (16.6)	13.0	77.7	25.5	84.1	23.7	88.2
202.5	27.6 (9.5)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
287.5	27.6 (9.5)	2.9	66.7	17.3	75.0	21.5	94.1
Toxic standard	100 (100)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] *corrected mortality* *n.d. not determined*

The study author claimed that exposure to fresh A-7402 G residues at concentrations up to and including 287.5 g as/ha had no significant effect on the mortality or reproduction of *Chrysoperla carnea.* Therefore, the author considered that NOEC was 287.5 g as/ha.

RMS comments:

The study was well performed and reported and is considered as valid for the risk assessment. No statistical analysis of the results was presented. Hence, it is difficult to conclude a reliable NOEC value from the fecundity data. However, it can be concluded that the ER_{50} value is >287.5 g as/ha. This value will be used in the further assessment.

Reference:	**Reber, B. (1999a). Toxicity of CGA 169374 EC 250 (A-7402 G) to the predator *Orius laevigatus* Fiber (Heteroptera: Anthocoridae) under extended laboratory conditions. Novartis Crop Protection AG, Basel, Switzerland. Unpublished report number 983928. (Syngenta No. CGA 169374/1965). 24 March – 3 May 1999.**
Guideline:	IOBC (1992); Escort (1994) and Candolfi and Vickus (1995).
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 G containing 253 g CGA 169374/L. Batch number P.612076.
Species:	Predator *Orius laevigatus*.
Treatments:	Maize (*Zea mays*) plants growing in pots (6 plants per pot, ca 10 cm tall) were sprayed with stock solutions of A-7402 G, prepared in deionised water, at a rate of 200 L/ha to give nominal exposure concentrations of 6.072, 30.36, 151.8 and 303.6 g as/ha. After air-drying, plants were transferred to untreated test chambers and 10 bugs (second instar nymphs) were introduced into each chamber.
Number of animals:	The test incorporated ten replicate chambers for each exposure concentration, ten replicate controls, in which plants were sprayed with deionised water, and ten replicates of a toxic standard in which plants were sprayed with ethyl parathion as an EC 200 formulation at 200 mL/ha. For the reproduction phase, 15 females from each exposure level was transferred to individual reproduction chambers (petri dishes, 3.5 cm in diameter, 1.3 cm in height).
Duration:	10 days exposure, 10 days reproduction.
Test conditions:	Chambers were maintained in a controlled environment at 25±2°C and a relative humidity of 70 – 76%, with a 16 hour day of 2500-3000 lux.
Observations:	After 10 days when all insects had reached the adult stage, the number of surviving bugs was recorded before transfer of females reproduction chambers. Egg numbers were counted after 3, 5, 7 and 10 days and females were transferred to clean chambers.
Data analysis:	Fisher exact test, Mann-Whitney U-test. Corrected mortality according to Abbot (1925).

Results:

Mortality and reproduction data are presented in Table B.9.5.2-3. Exposure to the toxic standard caused 100% mortality after 10 days. Exposure to A-7402 G at 6.072, 30.36 or 151.8 g as/ha did not have a significant effect

on the mortality of predatory bugs. However, for those bugs exposed to 303.6 g as/ha, mortality was significantly increased to 29% relative to 13% for control bugs. The number of eggs produced per female per day was not significantly affected by A-7402 G concentrations up to and including 303.6 g as/ha.

Table B.9.5.2-3: Effect of A-7402 G on mortality and reproduction in *Orius laevigatus*

Nominal concentration (g as/ha)	Mortality (%)[a]	Eggs/female/day
0	13 (0)	10.08
6.072	13 (0)	10.29
30.36	19 (6.9)	10.08
151.8	22 (10.3)	9.71
303.6	29* (18.4)	9.8
Toxic standard	98* (97.7)	n.d.

[a] *corrected mortality shown in brackets*
**significantly different from control*
n.d. not determined

Exposure to fresh residues of A-7402 G at concentrations up to and including 151.8 g as/ha, did not have a statistically significant effect on mortality or fecundity of *Orius laevigatus* bugs. Exposure to A-7402 G residues of 303.3 g as/ha caused 29% mortality but did not seem to affect the reproductive capacity of surviving females. Therefore, the NOEC was considered to be 151.8 g as/ha.

RMS comments:

The study was well performed and reported and is considered as valid for the risk assessment. The LR_{50} value can be concluded to be >304 g as/ha.

B.9.5.3 Semi-field studies

SCORE 250EC

Reference:	**Englehard, E.K. (1997a). CGA 169374 EC 250 (A-7402 G): Semi-field toxicity test with the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae). Springborn Laboratories (Europe), Horn, Switzerland. Unpublished report number 97-196-1047. (Syngenta No. CGA 169374/1466). 25 June – 19 August 1997.**
Guideline:	Mead-Briggs 1996.
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 G containing 248 g CGA 169374/L. Batch number 501026.
Species:	Cereal aphid parasitoid *Aphidius rhopalosiphi*.
Treatments:	A stock solution of A-7402 G, prepared in deionised water, was sprayed over wheat plots (2 x 10 m) at a rate of 481-492 L/ha to give a nominal exposure concentration of 125 g as/ha. Four applications of A-7402 G were made at 14-day intervals (from growth stage BBCH 32 to 69) and reproductive effects were assessed after the first and fourth applications. Immediately after each treatment, cage enclosures over a 1 m x 1m area in each plot were set up and the wasps were released into each cage. Two hours after application, potted barley plants, infested with aphids, were placed

	inside each enclosure. Pots were replaced daily for 3 days.
Number of replicates:	40 wasps (20 males and 20 females) were introduced into each enclosure. The test incorporated four replicate wheat plots for the A-7402 G concentration, four replicates of the control, which remained unsprayed, and four replicates of a toxic standard, which were sprayed with dimethoate (Perfekthion) at 0.1% by volume.
Duration:	Exposure for 2 days prior to introduction of barley plants with aphids for the 24 hour reproduction phase. Observations for 16 days.
Test conditions:	The exposure phase was conducted in wheat plots under field conditions and reproductive phase under laboratory conditions. **Exposure phase**: Temperature 8.3 – 30.1°C, precipitation 1.0 – 47 mm. **Reproduction phase**: Temperature 18 – 21.5°C, relative humidity 70 – 97%, light 16 hours per day, 2060 – 2750 Lux.
Observations:	Parasitoid behaviour was observed at 30 minutes and 2 hours after treatment in order to identify possible repellent effects. Numbers of parasitised aphids were recorded after 9-16 days.
Data analysis:	Shapiro-Wilk's test and t-test for fecundity data.

Results:

Reproduction data are presented in Table B.9.5.3-1. Observations of behaviour up to 2 hours after each application indicated that A-7402 G did not cause repellent effects in *Aphidius rhopalosiphi*. The mean number of aphid mummies produced per female after the first application was 3.19, 2.38 and 1.06 for control, A-7402 G and dimethoate-treated wheat plots, respectively. After the fourth application, wasps from control plots produced 4.5 mummies while those from A-7402 G-treated plots produced 3.17 mummies. Differences between control and A-7402 G treatment groups were not statistically significant following both applications.

Table B.9.5.3-1: Effect of A-7402 G on reproduction in *Aphidius rhopalosiphi*

Nominal concentration (g as/ha)	Mean number of aphid mummies per female	
	Application 1	Application 4
0	3.19±1.16	4.50±1.23
125	2.38±1.45	3.17±2.35
Toxic standard	1.06±1.01	0±0

Exposure of *Aphidius rhopalosiphi* adults to four applications of 125 g as/ha A-7402 G at 14-day intervals under field conditions did not have a statistically significant effect on aphid parasitisation rates measured after first and fourth applications.

RMS comments:

It was not completely clear whether the four subsequent treatments were performed on the same spot, since the enclosures were stated to be relocated within each plot between each exposure. If so, it could be questioned if this study would cover the representative use with four applications on the same field. Further, since the barley plants infested with aphids were not present in the field at treatment, the exposure levels would probably not

represent the worst case exposure conditions. The results are therefore not considered to be valid for this risk assessment.

Reference:	**Longley, M. (2001a). A semi-field study to evaluate the effects of fresh and aged residues of CGA 169374 EC 250 (A-7402 G) on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae). Ecotox Ltd., Tavistock, Devon, UK. Unpublished report number ER-00-KCB138. (Syngenta No. CGA 169374/2104). 30 June – 30 August 2000.**
Guideline:	ESCORT (Barrett et al, 1994).
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 G containing 263 g CGA 169374/L. Batch number WM002163.
Species:	Cereal aphid parasitoid *Aphidius rhopalosiphi*.
Treatments:	Beans were sown in seed trays and, on reaching the 4-6 leaf stage, were sprayed with stock solutions of A-7402 G, prepared in deionised water, at a rate of 400 L/ha to give nominal exposure concentrations of 75, 125 and 287.5 g as/ha. Plants were air-dried for 30 minutes before being individually sealed and transferred to a polythene tunnel in the field. On two occasions after spray application, (2.5 hours and 14 days), 40 wasps (20 males and 20 females) were introduced into each enclosure. Two hours after wasp introduction, a pot of barley plants, infested with aphids, was placed in the centre of each enclosure. After two days, the pot was replaced with a second pot, which was left in place for 2 days. Both pots were transferred to laboratory conditions at ambient temperature with a 16-hour day.
Number of animals:	The test incorporated 4 replicate trays (with 20 males and 20 females) for each A-7402 G concentration, four replicates of a control treatment, which were sprayed with tap water, and four replicates of a toxic standard which were sprayed with dimethoate at 0.84 L product/ha.
Duration:	Exposure to treated bean plants for 2 hours before introduction of barley plants with aphids for the 24 hours reproduction phase. Observations for 10 days.
Test conditions:	The exposure phase was conducted in broad beans under field conditions (8.0 – 42.6°C, relative hunidity 25 – 98%) and reproductive phase conducted under laboratory conditions (20 – 25°C, light intensity 1800 Lux).
Observations:	The number of parasitised aphids was recorded after 10 days.
Data analysis:	Two-way analysis of variance.

Results:

Numbers of mummies produced during each bioassay are listed in Table B.9.5.3-2. The data show that exposure to fresh residues of dimethoate, significantly reduced the number of aphid mummies produced per female from 6 in control wasps to 0.4 in those exposed to dimethoate. Similarly exposure to 14-day old dimethoate residues, significantly reduced mummy production from 5.9 per female in control wasps to 0.9 in dimethoate-exposed

wasps. Exposure to fresh or 14-day old residues of A-7402 G did not have a significant effect on mummy production.

Table B.9.5.3-2: Effect of A-7402 G on reproduction in *Aphidius rhopalosiphi*

Nominal concentration (g as/ha)	Mean number of aphid mummies per female	
	Bioassay 1 (Day 0)	**Bioassay 2 (Day 14)**
0	6.0	5.9
75	7.9	7.9
125	6.5	5.9
287.5	5.8	5.8
Toxic standard	0.4	0.9

Exposure of *Aphidius rhopalosiphi* adults to fresh or 14-day old residues of A-7402 G concentrations up to 287.5 g as/ha under field conditions did not have any significant effect on the production of aphid mummies.

RMS comments:

Since the barley plants infested with aphids were not present in the field at treatment, the exposure levels would probably not represent the worst case exposure conditions. The results are therefore not considered to be valid for this risk assessment.

Reference:	**Longley, M. (2001b). A semi-field study to evaluate the effects of fresh and aged residues of CGA 169374 EC 250 (A-7402 G) on the parasitic wasp, *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). Ecotox Ltd., Tavistock, Devon, UK. Unpublished report number ER-00-KCB141. (Syngenta No. CGA 169374/2105). 12 July – 18 September 2000.**
Guideline:	ESCORT (Barrett et al, 1994).
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 G containing 263 g CGA 169374/L. Batch number WM002163.
Species:	Parasitic wasp, *Trichogramma cacoeciae*.
Treatments:	The exposure phase was conducted in broad beans under field conditions and reproductive phase conducted under laboratory conditions. Beans were sown in seed trays and, on reaching the 4-6 leaf stage, were sprayed with stock solutions of A-7402 G, prepared in deionised water, at a rate of 400 L/ha to give nominal exposure concentrations of 15, 75, 125 and 287.5 g as/ha. Plants were transferred to a polyethene tunnel in the field and individually enclosed in a mesh tent. On two occasions after application, (3.5 hours and 14 days), adult wasps, which had newly emerged from parasitised *Sitotroga cerealella* eggs, were introduced into each enclosure. On two occasions (2 and 4 days) after each introduction, four circles of unparasitised *S. cerealella* eggs were introduced into each test unit for 24-hour periods.
Number of replicates:	The test incorporated 4 replicate trays for each A-7402 G concentration, four replicates of the control treatment, which was sprayed with tap water, and four

	replicates of a toxic standard, in which trays were sprayed with dimethoate (400 g/L) at 0.84 L/ha.
Duration:	2 – 5 days exposure, observation for further ca 12 days.
Test conditions:	In the field, temperature was 8.8 - 43°C, relative humidity 34 – 100%. Eight days after each introduction in the enclosure, eggs were transferred to a controlled environment chamber and maintained at 19-22°C and relative humidity 52 – 80%, with a 16-hour day of approximately 1450 Lux.
Observations:	*Sitotroga cerealella* eggs were monitored for signs of parasitisation and the number parasitised eggs was recorded.
Data analysis:	Student's t-test for bioassay 1, and ANOVA for bioassay 2.

Results:

The mean numbers of parasitised eggs produced per adult are presented in Table B.9.5.3-3. In both bioassays, exposure of adult parasitoids to the toxic standard caused 95-98% reduction in the number of parasitised eggs relative to control adults. In contrast, exposure to fresh or aged A-7402 G residues up to 287.5 g as/ha did not have any significant effect on the number of parasitised eggs per adult.

Table B.9.5.3-3: Effect of A-7402 G on reproduction in *Trichogramma cacoeciae*

Nominal concentration (g as/ha)	Mean number of parasitised eggs			
	Bioassay 1 (fresh residues)		Bioassay 2 (aged residues)	
	per egg circle	per adult	per egg circle	per adult
Control	384	1.2	798	4.3
15	343 (89%)	1.1 (92%)	689 (86%)	4.0 (93%)
75	393 (102%)	1.5 (125%)	710 (89%)	3.9 (91%)
125	405 (105%)	1.5 (125%	662 (83%)	3.8 (88%)
287.5	397 (103%)	1.3 (108%)	694 (87%)	3.4 (79%)
Toxic standard	7.5 (2%)	0.03 (2.5%)	38 (5%)	0.2 (4.7%)

The author of the study proposed that exposure to fresh or 14-day old residues of A-7402 G concentrations up to and including 287.5 g as/ha under semi-field conditions did not significantly affect the parasitic ability of *Trichogramma cacoeciae* adults.

RMS comments:

The study was well performed and reported. Although not statistically verified, there seemed to be an up to 21% reduction in parasitation rate (per egg circle) at the highest test concentration compared to the control in the aged residue study. In bioassay 1 (fresh residues) there was an increased number of parasitized eggs per adult, however, this is not considered as treatment related since no dose-response pattern could be observed. Hence, based on the reduced parasitation at the highest concentration (for aged residues) the NOEC can be set to 125 g as/ha, and the ER_{50} to >287.5 g as/ha. This value will be used in the further assessment.

Reference:	**Englehard, E.K. (1997b). CGA 169374 EC 250 (A-7402 G): Semi-field toxicity test with the seven-spotted lady beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). Springborn Laboratories (Europe), Horn,**

	Switzerland. Unpublished report number 97-198-1047. (Syngenta No. CGA 169374/1463). 12 June – 1 October 1997.
Guideline:	Schmuck et al (1997).
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 G containing 248 g CGA 169374/L. Batch number 501026.
Species:	Seven-spotted lady beetle, *Coccinella septempunctata* L.
Treatments:	A semi-field test with the exposure phase in broad beans (*Vicia faba*) under field conditions and reproductive phase under laboratory conditions was conducted. Beans were sown in seed trays and, on reaching the 2-leaf stage, were artificially infested with pea aphids as a food source. A stock solution of A-7402 G, prepared in deionised water, was sprayed over trays at a rate of 474-493 L/ha to give a nominal exposure concentration 125 g as/ha. Four applications of A-7402 G were made at 14-day intervals and beetle larvae were introduced prior to the first and fourth applications. Trays were sealed individually and transferred to a transparent PVC shelter in the field. Larvae were maintained under field conditions until pupation. Pupae and attached leaves were transferred to dishes and maintained in a controlled environment chamber until adult emergence. After recording pre-imaginal mortality, the surviving beetles from each treatment were pooled and ca 25 beetles from each treatment were transferred to oviposition cages. Egg production was assessed daily for up to 7 weeks and those eggs laid in weeks 3 to 5 were stored in petri-dishes for 10 days in order to assess hatching rate.
Number of replicates:	The test incorporated four replicate exposure trays with 20 beetles each for the A-7402 G concentration, four replicates of the control treatment, in which trays were sprayed with tap water, and four replicates of a toxic standard, in which trays were sprayed with pyrazophos (Afugan 30 EC) at 0.2 L/ha for applications 1 and 2, and 1.2 L/ha for applications 3 and 4. For the reproductive phase, 1 replicate per treatment was used, with ca 25 beetles per treatment.
Duration:	Exposure phase: until pupation of the larvae. Reproduction phase: observations up to 7 weeks.
Test conditions:	Field conditions (10 – 31°C, relative humidity 40 – 99%) during the exposure phase, laboratory conditions (controlled environment chamber at 22-25°C, relative humidity 59 – 84% and a 16-hour day of >1000 Lux). During the exposure phase and the reprodution phase, the beetles were fed daily with different aphid species.
Observations:	Pre-imaginal mortality, egg production, hatching rate.
Data analysis:	Corrected mortality according to Schneider-Orelli (1947). Data on pre-imaginal mortality, pupation and emergence rate was analysed by Yates corrected Chi-squared test. No statistical analyses of number of eggs and hatching rate were made, since only one replicate was used for the reproduction phase.

Results:

Mortality and reproduction data are presented in Table B.9.5.3-4. Exposure to the toxic standard caused 85 and 100% mortality after first and fourth applications, respectively. Mortality, pupation and emergence rates of *Coccinella septempunctata* were not statistically significantly affected by exposure to 125 g as/ha A-7402 G after first or fourth applications. For beetles exposed to A-7402 G, the number of eggs laid per female per day was 31% lower after the fourth application than for untreated beetles. The number of viable eggs laid per female per day was reduced by 15% after the first application and by 32% after the fourth application.

Table B.9.5.3-4: Effect of A-7402 G on mortality and reproduction in *Coccinella septempunctata*

Application number	Nominal concentration (g as/ha)	Mean mortality (%)[a]	Pupation (%)	Emergence (%)	Mean eggs/female/ day	Hatching rate (%)	Viable eggs per female per day.
1	0	37±14 (0)	67.5±17.6	95.4±6.0	5.70±2.68	92.4±9.77	79.3
	125	44±18 (12.6)	70.0±13.5	80.3±16.8	5.62±2.82	87.4±14.5	67.1
	Toxic standard	85±15 (76.3)	17.5±15.5	78.5±25.8	n.a.	n.a.	n.a.
4	0	11±6.3 (0)	88.8±6.3	100±0	3.43±3.85	81±23.9	73.0
	125	20±8.2 (9.9)	81.3±8.5	98.5±2.9	2.35±3.17	65.5±33.2	49.3
	Toxic standard	100±0 (100)	0±0	0±0	n.a.	n.a.	n.a.

[a] *corrected mortalities shown in brackets*
n.a. not applicable

RMS comments:

The study was well performed and reported. The high mortality in the control makes the results uncertain for the first application. Therefore, the results from the first application will not be used in the further risk assessment of difenoconazole. After the fourth application, there was still a large variation in the number of eggs laid and hatching rate. Hence, it is difficult to derive a NOEC value from this study. However, it can be concluded that the overall effects from after the fourth application are less than 50%. Hence, the ER_{50} from this study is considered to be >4 x 125 g as/ha.

Reference:	**Longley, M. (2001c). A semi-field study to evaluate the effects of fresh and aged residues of CGA 169374 EC 250 (A-7402 G) on the hoverfly, *Episyrphus balteatus* (Diptera: Syrphidae). Ecotox Ltd., Tavistock, Devon, UK. Unpublished report number ER-00-KCB139. (Syngenta No. CGA 169374/2132). 12 July – 13 November 2000.**
Guideline:	ESCORT (Barrett *et al*, 1994). Since no specific guideline is available for this species, the method was adapted from the guidelines for *Coccinella* sp.
GLP:	Yes.

Material and methods:

Test substance:	A-7402 G containing 263 g CGA 169374/L. Batch number WM002163.
Species:	Hoverfly, *Episyrphus balteatus*.
Treatments:	In bioassay 1 (fresh residues), broad beans were sown in trays under field conditions and on reaching the 4-6 leaf stage, were sprayed with stock solutions of A-7402 G, prepared in deionised water, at a rate of 400 L/ha to give nominal exposure concentrations of 15, 75, 125 and 287.5 g as/ha. After drying for 30 minutes, each tray was enclosed inside a mesh tent and aphids (>300) and twenty second instar hoverfly larvae were introduced into each test unit. When larvae began to pupate,

	test units were dismantled and numbers of surviving larvae and pupae were recorded. Due to low numbers of adults surviving under field conditions, further reproductive assessments were not possible and the exposure phase was repeated (bioassay 1-repeat). For this purpose, A-7402 G was applied to pot-grown beans at the 4-6 leaf stage under field conditions. Treated pots were enclosed in an acetate cylinder prior to the introduction of aphids (>200) and 10 second instar hoverfly larvae. Pots were then transferred to laboratory conditions and maintained at ambient temperature with a 16-hour day of approximately 2000 Lux. When larvae began to pupate, test units were dismantled and numbers of surviving larvae and pupae were recorded. On this occasion, emerged adults from control and 287.5 g as/ha treatments were transferred to reproduction chambers and maintained for 18 days prior to the introduction of a broad bean plant infested with aphids. This oviposition plant was replaced at 2-day intervals for up to 2 weeks. The eggs laid on each plant were counted and transferred to an environmental chamber to allow assessment of the number of larvae hatching over the following week. For bioassay 2, assessment of the effects of 14-day aged A-7402 G residues, the original treated plants were transplanted into pots and enclosed in acetate cylinders prior to the introduction of aphids and 10 hover fly larvae. Pots were then maintained under laboratory conditions and an assessment of reproductive effects was made as described previously.
Number of replicates:	The bioassay 1 test incorporated five replicate pots for each A-7402 G concentration, five replicates of a control treatment, which was sprayed with tap water, and two replicates of a toxic standard in which plants were sprayed with dimethoate (400 g/L) at 0.84 L/ha. The bioassay 2 test incorporated four replicate pots for each A-7402 G concentration, four replicates of the control treatment and two replicates of the toxic standard.
Duration:	Bioassay 1: Field conditions during treatments. Bioassay 1-repeat and bioassay 2: Treatments were applied under field conditions, pots were then transferred to laboratory conditions (see above).
Test conditions:	Exposure phase under field conditions: 7.5 – 39°C, relative humidity 30 – 100%. Reproduction phase under laboratory conditions: ca 20 – 28°C, relative humidity ca 40 – 65%.
Observations:	Numbers of surviving larvae and pupae, emergence of adults, number of eggs, number of eggs hatched and number of viable eggs per female.
Data analysis:	Student's t-test, one-way analysis of variance. Percentage data was arc-sin square root transformed prior to data analysis. Corrected mortality according to Abbot (1925).

The number of eggs laid per female and the percentage of eggs giving rise to larvae in the control and highest dose treatment were compared only qualitatively, due to the high species-inherent variability.

Results:

The effects of fresh and aged residues of A-7402 G on larval and pupal mortality, adult emergence and reproduction of *Episyrphus balteatus* are illustrated by data in the tables below. In all cases, exposure to the toxic standard caused 100% mortality. Exposure to fresh or aged residues of A-7402 G at 15-287.5 g as/ha did not have a statistically significant effect on the larval/pupal mortality or on adult emergence from the first generation.

The mean number of eggs laid per female (derived from the original larvae) per two days was 16.4 and 2.3 for control females and those hatching from larvae exposed to fresh A-7402 G residues of 287.5 g as/ha, respectively, and the number of these eggs that successfully hatched was 63.3 and 91.8%, respectively. When expressed as number of viable eggs per female, beetles exposed to fresh residues of A-7402 G laid 80% fewer eggs than untreated control beetles. In bioassay 2, control adults died before laying any eggs while those eggs laid by A-7402 G-treated females became desiccated before hatching was possible. Therefore, assessment of effects from aged residues on reproduction parameters was not possible.

Table B.9.5.3-5: Effect of A-7402 G on mortality in *Episyrphus balteatus*

Nominal concentration (g as/ha)	Larval/pupal mortality (%) (corrected mortality)			Adult emergence (%)		
	Bioassay 1 (fresh residues)	Bioassay 1 (repeat)	Bioassay 2 (14-day residues)	Bioassay 1 (fresh residues)	Bioassay 1 (repeat)	Bioassay 2 (14-day residues)
0	48.8	44.0	7.5	64.4	78.6	70.6
15	55.0 (12.1)	41.6 (0.0)	17.5 (10.8)	67.4	70.6	88.1
75	62.5 (26.8)	44.0 (0.0)	20.0 (13.5)	65.1	89.1	72.8
125	53.8 (9.8)	51.0 (10.7)	17.5 (10.8)	39.5	66.0	84.0
287.5	66.3 (34.2)	42.0 (0.0)	12.5 (5.4)	61.9	72.4	64.9
Toxic standard	100	100	100	n.d.	n.d.	n.d.

n.d. not determined

Table B.9.5.3-6: Effect of A-7402 G on reproduction in *Episyrphus balteatus*

Nominal concentration (g as/ha)	Bioassay 1 repeat (fresh residues)		
	Number eggs per female per 48 hours	Egg hatch success (%)	Total number viable eggs per female (14 days)
0	16.4 (0.0-50.8*)	63.3 (26.7-100)	67.3**
287.5	2.3 (0.0-7.7)	91.8 (65.2-100)	13.4

**50.8 was an outlier, the second higest value was 18.3 eggs per female per 48 hours. Excluding the highest value gave a mean value of 9.1 eggs per female per 48 hours.*
***The total number of viable eggs was 34.9 if the outlier value was excluded.*

Exposure of *Episyrphus balteatus* larvae to fresh or 14-day old residues of A-7402 G at rates up to and including 287.5 g as/ha, had no statistically significant effect on larval or pupal mortality. However, female hoverflies hatching from larvae exposed to fresh residues of 287.5 g as/ha A-7402 G laid 80% fewer viable eggs than untreated control flies. According to the study author, this data should be treated with caution due to a reported inherent variability in the reproductive performance of the test species under laboratory conditions and the high

levels of control mortalities reported in this test. Excluding an outlier value from the control treatment, would give an effect of 62% on the total number of viable eggs per female.

RMS comments:

The RMS agrees with the study author that the high mortality in the control of bioassay 1 makes the results uncertain. Also the results on reproduction showed a large variation within the control treatment. However, even if an outlier from the number of viable eggs per female per 48 hours was excluded from the control treatment (resulting in a total of 34.9 eggs per female, or 2.0 – 10.8 eggs per 48 hours), an effect of 62% could be observed in the treated group. Although these observed effects were above the trigger 50% for refinement, it should be kept in mind that the single dose tested were twice the highest recommended single dose, and only fresh residues were tested. It can be considered likely that at the lower normal use rates of difenoconazole the effects would be less pronounced and also that hoverflies that are assumed to be mobile would have a potential for re-colonisation. Hence, the risk for hoverflies is considered to be acceptable.

B.9.5.4 Field studies

Although risk assessments based on endpoints from laboratory tests indicate that A-7402 G poses a low risk to non-target arthropods, the following field studies were submitted to support the absence of adverse effects on predatory mites under field conditions.

Reference:	**Muther, J. (2000a). A field study to evaluate the effects of CGA 169374 EC 250 (A-7402 G) on predatory mites in an apple orchard in Italy. GAB Biotechnologie GmbH and IFU Umweltanalytik GmbH, D-75223 Niefern-Oschelbronn, Germany. Unpublished report number 20001049/Il-NFTp. (Syngenta No. CGA 169374/2097). 28 April – 3 July 2000.**
Guideline:	BBA-guideline Teil IV 23-2.4 (Heimann-Detlefsen, 1991); Draft Ringtest protocol (Blumel et al 2000); IOBC guideline (Boller et al 1988).
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 G containing 265 g CGA 169374/L. Batch number P.706093.
Species:	Predatory mites (Acari: Phytoseiidae). The majority was identified as *Kampimodromus aberrans*.
Treatments:	The field study was conducted in an apple orchard in Northern Italy between May and July 2000. A-7402 G (79.5 g as/ha for a crown height of 3 m) and a reference compound (Decis, 9 g deltamethrin/ha) were applied at a rate of 500 L/ha per metre of crown height, using a knapsack sprayer. Four applications of A-7402 G and the water control were made at 10 or 11-day intervals between May 3 and June 5.
Number of replicates:	The study incorporated 5 replicate rows of 6 trees for A-7402 G and the control, which was sprayed with water, and 4 replicate rows of 6 trees for the reference compound. A single application of the reference compound was applied on two occasions coinciding with the second (Decis 1) and fourth (Decis 2) applications of A-7402 G, to 2 replicate rows of 6 trees. Different replicate plots were treated on

	each occasion.
Duration:	Samples were taken for analysis up to 28 days after the last treatment.
Test conditions:	Field conditions in northern Italy.
Observations:	The number of predatory mites and spider mites were assessed 2 days prior to the first application, immediately before the second, third and fourth applications and 7 and 28 days after the final application. On the first two sampling occasions, 50 leaves were selected randomly from the upper, middle and lower parts of the central four trees in each replicate plot. On subsequent occasions, 25 leaves were collected. Mites were then rinsed from the leaf samples with a water-detergent solution and immersed in 70% ethanol before staining with methylene-blue to allow distinction between the mites and remnant plant tissue.
Data analysis:	The numbers of mites per treatment and plot were square-root transformed, homogeneity of variance was checked by Shapiro Wilks test, and statistically significant differences were determined by Dunnett's test. The corrected effects compared to the control values were calculated according to Abbot (1925).

Results:

Mite population data is presented in Table B.9.5.4-1. Data collected 59 days after the first application showed that mite density was significantly increased compared to the control by approximately 300% following exposure to A-7402 G. In samples from other timepoints, A-7402 G treatments showed no significant effects on numbers of predatory mites compared to the control. Treatment with the first single application of the toxic standard (Decis 1) caused significant reductions in mite densities recorded on the three sampling occasions after 10, 15 and 21 days (20, 31 and 38 days after the first A-7402 G application). In plots treated with the second application of the toxic standard (Decis 2), there was a non-significant reduction in mite density of up to 65% at the subsequent sampling occasions (38 and 59 days after the first A-7402 G application).

Table B.9.5.4-1: Effect of A-7402 G on predatory mites in an apple orchard

Time after first application (days)	Date/Action	Mean number of predatory mites per leaf			
		Control	A-7402 G	Decis 1	Decis 2
-2	03/05/00: Before 1st appl.	0.67±0.18	0.78±0.41	0.77±0.18	0.60±0.14
0	05/05/00: 1st appl. A-7402 G	no samples	no samples	no samples	no samples
10	15/05/00: 2nd appl. A-7402 G and Decis 1	1.86±0.44	1.82±0.60	1.90±0.03	1.40±0.40
20	25/05/00 3rd appln of A-7402 G	4.08±1.03	2.66±0.97	0.84±0.28*	3.84±1.41
31	05/06/00: 4th appl. A-7402 G and Decis 2	6.84±0.86	5.94±0.76	0.22±0.03*	6.86±0.08
38	12/06/00	2.94±0.52	4.50±1.26	0.28±0.23*	1.30±1.05
59	03/07/00	0.91±1.07	2.79±0.90*	0.44±0.28	0.32±0.28

**significantly different from untreated value on same occasion ($p \leq 0.05$).*

Treatment of apple trees with 4 applications of A-7402 G (79.5 g as/ha) at intervals of 10 or 11 days did not have any significant negative effect on the population density of predatory mites recorded up to 28 days after the last application.

RMS comments:

The study was well performed and reported and is considered as valid for the risk assessment.

Reference:	**Muther, J. (2000b). A field study to evaluate the effects of CGA 169374 EC 250 (A-7402 G) on predatory mites in an apple orchard in Germany. GAB Biotechnologie GmbH and IFU Umweltanalytik GmbH, D-75223 Niefern-Oschelbronn, Germany. Unpublished report number 20001049/Gl-NFTp. (Syngenta No. CGA 169374/2096). 28 April – 3 July 2000.**
Guideline:	BBA-guideline Teil IV 23-2.4 (Heimann-Detlefsen, 1991); Draft Ringtest protocol (Blumel et al 2000); IOBC guideline (Boller et al 1988).
GLP:	Yes.

Material and methods:

Test substance:	A-7402 G containing 265 g CGA 169374/L. Batch number P.706093.
Species:	Predatory mites (Acari: Phytoseiidae)
Treatments:	The field study was conducted in an apple orchard in South-west Germany between May and July 2000. A-7402 G (59.6 g as/ha for a crown height of 3 m) and a reference compound (Decis, 6.8 g deltamethrin/ha) were applied at a rate of 500L/ha per metre crown height, using a knapsack sprayer. Four applications of A-7402 G and the water control were made at 9-12 day intervals between May 2 and June 2. The reference compound was applied on two occasions coinciding with the second (Decis 1) and fourth (Decis 2) applications of A-7402 G, to 2 replicate rows of 8 trees. Different replicates were treated on each occasion.
Number of replicates:	The study incorporated 5 replicate rows of 8 trees for A-7402 G and the control, which was sprayed with water, and 4 replicate rows of 8 trees for the reference compound.
Duration:	Samples were taken for analysis up to 28 days after the last treatment.
Test conditions:	Field conditions in south-west Germany.
Observations:	The number of predatory mites and spider mites were assessed 4 days prior to the first application, up to 1 day before the second, third and fourth applications and 6 and 27 days after the final application. On the first sampling occasion, 100 leaves were selected randomly from the upper, middle and lower parts of the central six trees in each replicate plot. On subsequent occasions, 25 leaves were collected. Mites were then rinsed from the leaf samples with a water-detergent solution and immersed in 70% ethanol before staining with methylene-blue to allow distinction between the mites and remnant plant tissue.
Data analysis:	The numbers of mites per treatment and plot were square-root transformed, homogeneity of variance was checked by Shapiro Wilks test, and statistically significant differences were determined by Dunnett's test. The corrected effects compared to the control values were calculated according to Abbot (1925).

Results:

Mite population data is presented in Table B.9.5.4-2. Treatment with A-7402 G did not cause any significant effects on numbers of predatory mites recorded during the study. Treatment with a single application of the toxic reference (Decis 1) caused significant reductions of up to 93% in mite densities in the samples taken after 10 to 48 days (27 to 58 days after the first application fo A-7402 G). The second application of the toxic reference (Decis 2) caused significant reductions of up to 98% in mite density measured in the samples taken after 6 and 27 days (37 and 58 days after the first application of A-7402 G).

Table B.9.5.4-2: Effect of A-7402 G on predatory mites in an apple orchard

Time after first application (days)	Date/Action	Mean number of predatory mites per leaf			
		Control	A-7402 G	Decis 1	Decis 2
-4	28/04/00: Before 1st appl.	0.19±0.08	0.19±0.08	0.16±0.0	0.15±0.06
0	02/05/00: 1st appl. A-7402 G	no samples	no samples	no samples	no samples
10	11/05/00: 2nd appl. A-7402 G & Decis 1	3.20±1.08	2.50±0.25	4.22±1.27	3.28±0.45
20	22/05/00	2.38±0.38	1.86±0.53	0.52±0.11*	2.04±0.06
21	23/05/00: 3rd appl. A-7402 G	no samples	no samples	no samples	no samples
31	02/06/00: 4th appl. A-7402 G & Decis 2	2.30±0.96	1.86±0.50	0.24±0.28*	1.28±0.17
37	08/06/00	2.04±0.44	1.66±0.47	0.16±0.11*	0.22±0.03*
58	29/06/00	2.15±0.22	1.65±0.71	0.16±0.0*	0.04±0.0*

**significantly different from untreated value on same occasion ($p \leq 0.05$).*

Treatment of apple trees with 4 applications of A-7402 G (59.6 g as/ha) at intervals of 9-12 days did not result in statistically significant effects on the population density of predatory mites recorded up to 28 days after the last application.

RMS comments:

The study was well performed and reported and is considered as valid for the risk assessment.

B.9.5.5 Summary and risk assessment for non-target arthropod species other than bees.

A summary of the results from available studies on non-target arthropods is given in the table below.

Table B.9.5.5-1: Summary of results from non-target arthropod studies with difenoconazole and representative formulations.

Species	Test type	Rate (g as/ha)	Result	Reference
LABORATORY STUDIES				
ACTIVE INGREDIENT				
Aphidius rhopalosiphi	fresh residues on glass plates.	18, 36, 72, 144, 288	LR_{50} 178 g as/ha	Kleiner (2000a)
Typhlodromus pyri	fresh residues on glass plates.	18, 36, 72, 144, 288	LR_{50} 112 g as/ha	Kleiner (2001)
DIVIDEND 030FS				
Aleochara bilineata	treated seeds in moistened sand	23.2 g as/ha (60 mg as/kg seed and seed density of 379 kg/ha	LR_{50} >23.2 g as/ha	Grimm (1999a)
Poecilus cupreus	treated seeds in moistened sand	18.8 g as/ha (60 mg as/kg seed and seed density of 307 kg/ha	LR_{50} >18.8 g as/ha	Grimm (1999b)
Poecilus cupreus	treated seeds in moistened sand	56.4 g as/ha (60 mg/kg seed and seed density of 937 kg/ha	LR_{50} >56.4 g as/ha	Reber (1999)
SCORE 250EC				
Aphidius rhopalosiphi	fresh residues on glass plates.	5, 127, 253	ER_{50} 127 - 253 g as/ha	Nienstedt (1999a)
Typhlodromus pyri	fresh residues on glass plates.	5, 127, 253	ER_{50} 127 - 253 g as/ha	Nienstedt (1999b)
Chrysoperla carnea	fresh residues on glass plates	4, 100, 200	ER_{50} >200 g as/ha	Kleiner (2000b)
Pardosa spp.	direct spray over adults, food and substrate (sand).	4, 100, 200	ER_{50} >200 g as/ha	Kleiner (1999)
Poecilus cupreus	direct spray over adults, food and substrate (sand).	6, 30, 150, 300	ER_{50} >300 g as/ha	Reber (1999b)
EXTENDED LABORATORY STUDIES				
SCORE 250EC				
Typhlodromus pyri	fresh residues on bean leaves	6, 30, 150, 300	ER_{50} 152 - 303 g as/ha	Grimm (1999)
Chrysoperla carnea	fresh residues on bean leaves	14, 28, 75, 125, 202, 288	ER_{50} >288 g as/ha	Walker (2001)
Orius laevigatus	fresh residues on maize plants	6, 30, 150, 300	ER_{50} >300 g as/ha	Reber (1999a)
SEMI-FIELD STUDIES				
SCORE 250EC				
Aphidius rhopalosiphi	fresh and 14-day old residues on broad beans.	75, 125, 288	ER_{50} >288 g as/ha.	Longley (2001a)
Trichogramma cacoeciae	fresh and 14-day old residues on broad beans.	15, 75, 125, 288	ER_{50} >288 g as/ha	Longley (2001b)
Coccinella septempunctata	fresh and 14-day aged residues on broad beans.	4 applications of 125 g as/ha at 14-day intervals	ER_{50} >4 x 125 g as/ha	Engelhard (1997b)
Episyrphus balteatus	14-day aged residues on broad beans.	15, 75, 125, 288	ER_{50} >288 g as/ha for aged residues based on mortality. Results from fresh residues not reliable due to high control mortality.	Longley (2001c)
Episyrphus balteatus	fresh residues on broad beans.	288	62% effect on number of viable eggs per female when an outlier was excluded. Aged residues not tested for reproduction. Potential for recovery is considered likely.	Longley (2001c)

Species	Test type	Rate (g as/ha)	Result	Reference
FIELD STUDIES				
SCORE 250EC				
Predatory mites	Field study on in apple orchards in Italy.	4 applications of 79.5 g as/ha at intervals of 10 or 11 days	No significant effect on the population density of predatory mites up to 28 days after the last application, except for an increased population on day 28 after the last application.	Muther (2000a)
Predatory mites	Field study in apple orchards in Italy.	4 applications of 59.6 g as/ha at intervals of 10 or 11 days	No significant effect on the population density of predatory mites up to 28 days after the last application.	Muther (2000b)

The available studies are considered to fulfil the data requirements of Annex II and III of 91/414, and are sufficient for the risk assessment for non-target arthropods.

B.9.5.5.1 SEED TREATMENT WITH DIVIDEND 030FS

A risk assessment for non-target arthropods at seed treatment with DIVIDEND 030FS was provided by the notifier in **Document M-III, Section 6**. A summary is given below.

Non-target arthropods living in the crop may be exposed to seed-treatments, such as DIVIDEND 030FS, through direct contact with residues in the soil or on treated seed, or by feeding on crop plants containing difenoconazole residues. The maximum residual concentration of difenoconazole in soil and foliage was assumed to be equivalent to the proposed seed-treatment rate of 6g as/100 kg seed at a seed density of 205 kg wheat seed/ha (equivalent to 12.3 g as/ha).

As DIVIDEND 030FS is a seed-treatment that is applied directly to the crop seed prior to sowing, non-target arthropods living in off-crop areas are unlikely to be exposed to this pesticide and will not be considered in this risk assessment.

Hazard quotients for non-target arthropods exposed within the treated area were calculated according to the following equation.

$$\text{In - crop HQ} = \frac{\text{PER (g ai/ha)}}{\text{LR}_{50}\text{ (g ai/ha)}}$$

HQ values, calculated using LR_{50} values from glass-plate, dose response tests conducted with the standard sensitive species *Aphidius rhopalosiphi* and *Typhlodromas pyri,* are presented in the table below. All HQ values fall below the ESCORT 2 trigger value of 2 for Tier 1 studies on inert substrates thus indicating that difenoconazole poses a low risk to non-target arthropods within the crop following the proposed use for seed treatment.

Table B.9.5.5-2: In-crop hazard quotients for non-target arthropod standard sensitive species at seed treatment with DIVIDEND 030FS.

Species	LR_{50} (g as/ha)	PER (g as/ha)	HQ
A. rhopalosiphi	178	12.3	0.069
T. pyri	112	12.3	0.11

Non-target arthropod tests were also conducted with representative ground-dwelling species (*Aleochara bilineata* and *Poecilus cupreus*) exposed to wheat seeds treated with DIVIDEND 030FS at 2 mL/kg seed at seed densities up to 937 kg/ha (equivalent to a maximum of 56.4 g as/ha). Exposure to DIVIDEND 030FS did not cause a significant effect on the mortality or fecundity of those species tested, at rates up to 4.5-fold greater than the maximum predicted exposure rate of 12.3 g as/ha.

RMS comments

The RMS agrees with the assumptions and conclusions proposed by the notifier. In conclusion, the risk for non-target arthropods is considered to be low at the use of difenoconazole for seed treatment. No further refinement is needed.

B.9.5.5.2 SPRAY APPLICATION WITH SCORE 250EC

A risk assessment for non-target arthropods at spray application with SCORE 250EC was provided by the notifier in **Document M-III, Section 6.** A summary with comments from RMS and slightly modified calculations are given below.

In-field

Non-target arthropods living in the crop may be exposed to SCORE 250EC by direct over-spray during spray operations, through contact with residues on plants and soil, or by feeding on exposed food items. For foliar residues, the notifier assumed that 100% of the spray is deposited on the crop with a foliar half-life of 7.7 days. Since there is no clear justification for the foliar half-life, the RMS has chosen to use the default DT_{50} of 10 days. For soil residues, 65 and 80% of spray was assumed to be intercepted in pome fruit and carrot crops, respectively. The remaining spray was assumed to reach the soil surface and have a soil half-life of 179 days (based on notifier's assessment in **Document M-III, Section 5, Point 9.1**). Based on the RMS evaluation of soil data (Annex B.8), a soil DT_{50} of 246 days is considered to be more appropriate for the exposure assessment. Assuming first-order degradation, the maximum predicted environmental residues (PER) for foliage and soil, after *n* applications with *i* day intervals were calculated according to the following equation:

$$\text{Maximum in - field PER} = \frac{\text{application rate (g ai/ha) x } (1 - e^{-nki})}{(1 - e^{-ki})}$$

where *k* is the dissipation constant given by

$$k = \frac{\ln(2)}{\text{half - life in days}}$$

n = number of treatments, i = interval between treatments

The maximum in-crop soil and foliar PER values for each use pattern are presented in the table below. The corrected figures according to the RMS comments above (foliar DT_{50} 10 days, soil DT_{50} 246 days) are given in parentheses.

Table B.9.5.5-3: In crop soil and foliar PER values at spray application with SCORE 250EC. Calculated by the notifier and RMS (within parentheses).

Crop	Application rate (g as/ha)	Number of applications	Application interval (days)	PER (g as/ha)	
				In-crop soil	In-crop foliar
Pome fruit	75	4	7	101 (102)	148 (167)
Carrots	125	3	14	71 (72)	170 (190)

Hazard quotients for non-target arthropods exposed within the sprayed area were calculated according to the following equation.

$$\text{In - field HQ} = \frac{\text{In - field PER (g ai/ha)}}{\text{LR}_{50}\text{(g ai/ha)}}$$

HQ values, calculated using LR_{50} values from glass-plate, dose response tests conducted with the standard sensitive species *Aphidius rhopalosiphi* and *Typhlodromus pyri,* based on the PER values corrected by the RMS are presented in the table below. All HQ values fall below the ESCORT 2 trigger value of 2 for Tier 1 studies on inert substrates thus indicating that SCORE 250EC poses a low risk to non-target arthropods within the crop.

Table B.9.5.5-4: In-crop hazard quotients for non-target arthropod standard sensitive species at spray application with SCORE 250EC. Based on PER values corrected by the RMS.

Use pattern	Species	LR_{50} (g as/ha)	In-crop soil		In-crop foliar	
			PER (g as/ha)	HQ	PER (g as/ha)	HQ
Pome fruit	*A. rhopalosiphi*	178	102	0.57	167	0.94
	T. pyri	112	102	0.91	167	1.49
Carrots	*A. rhopalosiphi*	178	72	0.40	190	1.1
	T. pyri	112	72	0.64	190	1.7

Off field

Non-target arthropods living in off-crop areas may be exposed to SCORE 250EC by spray drift from field applications. Off-crop areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure *via* soil residues in off-crop areas was not considered. Off-crop foliar PER values were calculated from in-crop foliar PERs in conjunction with BBA drift values (cited in ESCORT 2; 10.12% at 3 m distance for 4 applications to pome fruit and 2.01% at 1 m distance for 3 applications to field crops (carrots) as shown in the following equation:

$$\text{Off - field foliar PER} = \frac{\text{Maximum in - field foliar PER x (\% drift/100)}}{\text{vegetation distribution factor}}$$

The model used to estimate spray drift was developed for drift onto a two-dimensional water surface and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas. Therefore,

a vegetation distribution or dilution factor of 10 is incorporated into the equation when calculating PERs to be used in conjunction with toxicity endpoints derived from two-dimensional, glass plate or leaf disc studies. Maximum off-crop foliar PERs for each use pattern were calculated at distances of 1m for carrots and 3 m for pome fruit. PERs were estimated assuming 65% foliar interception for pome fruit. Resulting values are shown in the table below.

Table B.9.5.5-5: Maximum off-crop PER values at spray application with SCORE 250EC. Calculated by the notifier and RMS (within parentheses).

Crop	Application rate (g as/ha)	Number of applications	Application interval (days)	PER (g as/ha)	
				1 m from crop	3 m from crop
Pome fruit	75	4	7	n.d.	1.49 (1.69)
Carrots	125	3	14	0.34 (0.38)	n.d.

n.d. not determined

Hazard quotients for non-target arthropods exposed in off-crop areas were calculated according to the following equation.

$$\text{Off - field HQ} = \frac{\text{Off - field PER(g ai/ha)}}{\text{LR}_{50}\text{(gai/ha)}} \times \text{correction factor}$$

When calculating HQs using toxicity data from Tier 1 lab studies with the standard two species, a correction factor of 10 is incorporated to allow for extrapolation to the species diversity expected in off-crop areas. HQ values, calculated using LR_{50} values from glass-plate, dose response tests conducted with the standard sensitive species *Aphidius rhopalosiphi* and *Typhlodromus pyri*, and based on the off-field PER-values corrected by the RMS are presented in the table below. All HQ values fall below the ESCORT 2 trigger value of 2 for Tier 1 studies on inert substrates thus indicating that SCORE 250EC poses a low risk to non-target arthropods in off-crop areas.

Table B.9.5.5-6: Off-crop hazard quotients for non-target arthropod standard sensitive species at spray application with SCORE 250EC. Based on PER values corrected by the RMS.

Use pattern	Species	LR_{50} (g as/ha)	Distance from crop (m)			
			1		3	
			PER (g as/ha)	HQ	PER (g as/ha)	HQ
Pome fruit	*A. rhopalosiphi*	178	n.d.	n.d.	1.69	0.094
	T. pyri	112	n.d.	n.d.	1.69	0.15
Carrots	*A. rhopalosiphi*	178	0.38	0.021	n.d.	n.d.
	T. pyri	112	0.38	0.034	n.d.	n.d.

n.d. not determined

Further support of low risk to non-target arthropods was provided by additional laboratory and semi-field studies with a range of species including parasitoids, predatory mites, foliage-dwellers and ground-dwellers. In lab tests, effects on mortality or fecundity of >50% were not reported following a single application of 125 g as/ha. Similarly, results from semi-field studies where four applications of A-7402 G at 125 g as/ha were made at 14-day intervals, indicated that exposure to dried residues after four applications, did not cause >50% effect on mortality or fecundity in those species tested. As stated in the **Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002, October 2002)**, effects of <50% seen in higher-tier studies are considered

acceptable provided that the test covered the appropriate field rate. Therefore, SCORE 250EC is considered to pose low risk to non-target arthropods when applied in accordance with proposed representative use patterns in pome fruit and carrots.

The RMS agrees with the overall conclusion of the risk assessment proposed by the notifier, that the risk to in-crop and off-crop populations of non-target arthropods is low at the representative spray application with SCORE 250EC. No further data is needed.

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

B.9.6.1 Acute toxicity

ACTIVE INGREDIENT

Reference:	**Surprenant, D.C. (1987c). Fourteen-day toxicity test exposing earthworm (*Eisenia foetida*) to CGA 169374. Springborn Life Sciences Inc., USA. Unpublished report no. 87-9-2494. (Syngenta File No 169374/0027)**
Guideline:	OECD 207 (1984).
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number FL 85406, purity 96.1%; ^{14}C-labelled difenoconazole, specific activity 19.2 µCi/mg.
Species:	*Eisenia foetida*
Treatments:	^{14}C-labelled difenoconazole was prepared in acetone and mixed with artificial soil to give nominal concentrations of 31, 63, 130, 250 and 500 mg/kg soil.
Number of animals:	Four replicate test vessels of 10 worms for each exposure concentration, four replicates of a solvent control prepared with acetone (25 mL/kg) and four replicates of an untreated control.
Duration:	14 days.
Test conditions:	Temperature 21 – 22°C, soil moisture 20 - 23%. The vessels were kept in darkness.
Observations:	Observations of earthworm mortality were made on day 7 and 14 while weight was recorded at test initiation and termination. Soil samples were collected immediately prior to test initiation for analysis of test substance concentration by combustion and LSC.
Data analysis:	ANOVA and William's test.

Results:

Analysis of soil samples taken prior to test initiation showed that measured concentrations of difenoconazole were 44, 79, 170, 300 and 610 mg/kg soil. Mortality and weight data are presented in the table below. All worms suffered weight loss during the 14-day exposure period although those worms exposed to 610 mg/kg suffered a significantly greater reduction than untreated worms. However, exposure to difenoconazole at concentrations up to 610 mg/kg soil did not significantly affect earthworm mortality.

Table B.9.6.1-1: Effect of difenoconazole on earthworm mortality.

Mean measured concentration (mg/kg soil)	Cumulative mortality (total number out of 40 earthworms)		Mean weight reduction over test duration (%)
	Day 7	Day 14	
Control	0	1	26
Solvent control	2	2	20
44	0	0	25
79	0	2	20
170	0	0	29
300	1	1	12
610	0	1	30*

**significantly different from control ($p \leq 0.05$)*

As mortality did not reach 50% at any of the concentrations tested, the 14-day LC_{50} for difenoconazole in earthworms was considered to be >610 mg/kg soil.

RMS comments:

The study was conducted in accordance with the referred guidelines and is considered as valid for the risk assessment.

METABOLITES

Reference:	**Heimbach F (1986). Acute toxicity of 1,2,4-triazole (technical) to earthworms. Bayer, AG, Germany. Unpublished report no. HBF/Rg 59. (Syngenta File No 71019/0021)**
Guideline:	OECD 207 (1984).
GLP:	Yes.
Material and methods:	
Test substance:	Technical CGA 71019, Batch No. 301/84; Purity 95.8%.
Species:	*Eisenia foetida andrei*
Treatments:	In a preliminary range-finding test, adult earthworms (*Eisenia foetida;* mean weight 405 mg) were exposed for 14 days to CGA 71019 at concentrations of 0.1, 1.0, 10, 100 and 1000 mg/kg soil (dry weight) and to the toxic standard chloracetamide at concentrations of 10, 18, 24, 32 and 56 mg/kg soil (dry weight) in artificial soil. In a subsequent test, adult worms (mean weight 360 mg) were exposed to 1000 mg/kg CGA 71019 for 14 days.
Number of animals:	The pre-test incorporated two replicate chambers of ten adult worms for each test concentration and for the untreated control. The main test incorporated four replicate chambers of ten adult worms for the test substance treatment and for the untreated control.
Duration:	14 days.
Test conditions:	Temperature 20±2°C, soil pH 5.9 – 6.5, moisture 69 – 83% of maximum water capacity. Continuous light. The worms were not fed during the study.
Observations:	Temperature, pH and soil moisture content were recorded during the study. Assessments of mortality, effects on bodyweight and symptoms of toxicity were

made at days 7 and 14 in both tests.

Data analysis: Not stated, not needed.

Results:

Based on the study data, the LC_{50} for the toxic standard was calculated to be 24.4 mg/kg, thus confirming that the sensitivity of the test system was acceptable. Effects of CGA 71019 on mortality and bodyweight data are presented in the table below. No mortalities were recorded in the pre-test or the main test at the highest concentration tested (1000 mg CGA 71019/kg). Exposure to 1000 mg/kg in both the pre- and main tests caused a 12 – 14% reduction in bodyweight compared to controls. The 14-day NOEC was 100 mg CGA 71019/kg soil.

Table B.9.6.1-2: Effect of CGA 71019 on earthworm mortality and body weight

Nominal concentration [mg/kg soil]	Mortality-day 14 [%]	Bodyweight increase day 14 [%]
Pre-test		
Control	0	1±0
0.1	0	0±0
1.0	0	4±0
10	0	6±1
100	0	2±0
1000	0	-14±4
Main test		
Control	0	17±5
1000	0	-12±3

Based on nominal concentrations, the 14-day LC_{50} of CGA 71019 for earthworms was >1000 mg/kg soil.

RMS comments:

No analytical measurements of the substrate were reported, and therefore the test concentrations are uncertain. However, since analysis are not required in the referred guidelines, and the properties of the test compound do not indicate a potential for rapid degradation or volatilisation, the study is considered to be valid.

Reference: **Batscher, R. (2002). Acute toxicity of CGA 205375 (metabolite of CGA 169374) to the earthworm *Eisenia fetida* in a 14-day test. RCC Ltd., Switzerland. Unpublished report no. 812092. (Syngenta File No 205375/0011)**

Guideline: OECD 207 (1984); EU 87/302/EEC, L133.

GLP: Yes.

Material and methods:

Test substance: CGA 205375, batch number MLA-421/2, purity 99%.

Species: *Eisenia fetida.*

Treatments: Worms were exposed to CGA 205375 prepared in acetone and mixed with artificial soil to give nominal concentrations of 100, 178, 316, 562 and 1000 mg/kg soil.

Number of animals: The test incorporated 4 replicate test vessels of 10 worms for each exposure concentration and a control treatment, prepared with acetone.

Duration: 14 days.

Test conditions: Temperature 20 - 22°C, soil pH 6.4 – 6.5, moisture 33 - 34%. Continuous light.

Observations:	Observations of earthworm mortality were made on day 7 and 14 while weight was recorded at test initiation and termination.
Data analysis:	Probit analysis of the mortality data, multiple Dunnett's test with the body weight data.

Results:

Earthworm mortality and weight data are presented in Table 8.4.1.3. All worms suffered some weight loss during the 14-day exposure period although those exposed to CGA 205375 did not experience significantly greater reductions than untreated worms. Exposure to CGA 205375 concentrations up to 178 mg/kg did not significant effect on earthworm mortality. However, exposure to 316 and 562 caused 45 and 100%, mortality by day 14 while exposure to 1000 mg/kg caused 100% mortality by day 7.

Table B.9.6.1-3: Effect of CGA 205375 on earthworm mortality and weight.

Nominal concentration (mg/kg soil)	Cumulative mortality (%)		Mean weight reduction over test duration (%)
	Day 7	Day 14	
Control + acetone	0	0	4
100	0	0	7
178	5	5	10
316	10	45	9
562	65	100	n.a.
1000	100	n.a.	n.a.

n.a. not applicable

As exposure to CGA 205375 concentrations up to 178 mg/kg did not have any significant effects on *Eisenia fetida* after 14 days, the NOEC was considered to be 178 mg /kg. The LC_{50} was estimated to be 312 mg/kg with 95% confidence limits ($p \leq 0.05$) of 284-343 mg/kg.

RMS comments:

The study was conducted in accordance with the referred guidelines. No analytical measurements of the substrate were reported, and therefore the test concentrations are uncertain. However, since analysis are not required in the referred guidelines, and the properties of the test compound do not indicate a potential for rapid degradation or volatilisation, the study is considered to be valid.

FORMULATED PRODUCTS

DIVIDEND 030FS

No study was submitted on the acute toxicity of DIVIDEND 030FS to earthworms. Instead, a 56 days study on sublethal effects and reproduction is available, which is considered to cover also the short term toxicity of this formulation. The study (Friedrich, 2002) is summarised in Section B.9.6.2. Since no mortality was observed at the highest test concentration (0.4 mg as/kg soil) in that study, the LC_{50} can be concluded to be >0.4 kg as/kg. This value will be used in the short term risk assessment for DIVIDEND 030FS.

SCORE 250EC

Reference:	**Thun, S. (1993). Acute toxicity in earthworms according to OECD 207. Test article CGD 94630F. IBR Forschungs GmbH, Hannover, Germany. Unpublished report number 80-91-0423-06-90. (Syngenta No. CGA 169374/0763). 7-21 October 1993.**
Guideline:	OECD 207.
GLP:	Yes.

Material and methods:

Test substance:	CGD 96430 F (equivalent to A-7402 A) containing 250 g CGA 169374/L. Batch number P 111003.
Species:	*Eisenia foetida*
Treatments:	Stock solutions of A-7402 A prepared in deionised water were mixed into artificial soil to give nominal test concentrations of 56, 100, 180, 320 and 560 mg formulation/kg soil. Soil was transferred to test chambers and adult earthworms (mean weight 398 mg) were introduced into each chamber.
Number of animals:	The test incorporated four replicate chambers with ten worms for each exposure concentration and four replicate vessels for a control treatment, prepared by mixing soil with water.
Duration:	14 days.
Test conditions:	Test chambers were maintained at 19 - 21°C with continuous light of 600 lux. Soil pH was 6.5±0.5 and moisture 34 - 40%.
Observations:	Worms were weighed prior to test initiation and after 14 days. Adult worms were removed from substrate on days 7 and 14 for assessment of mortality and health.
Data analysis:	Probit analysis according to Spearman-Kärber.

Results:

Mortality and weight data are presented in the table below. Exposure to A-7402 A concentrations of 56, 100, 180, 320 and 560 mg/kg caused 0, 5, 65, 100 and 100% mortality by day 14. On day 7, worms exposed to 320 mg/kg appeared pale, thin and lethargic. Similar symptoms were seen in worms exposed to 180 mg/kg on day 14. Surviving worms exposed to 100 mg/kg did not exhibit these symptoms. All worms suffered some weight loss during the 14-day exposure phase and those exposed to A-7402 A concentrations of 100 and 180 mg/kg suffered 8 and 80% greater weight loss than control worms.

Table B.9.6.1-4: Effect of A-7402 A on earthworm mortality and weight

Nominal concentration (mg formulation/kg)	Mortality (%)	Mean weight decrease (day 0- day 14; mg)
0	0	95
56	0	86
100	5	103
180	65	170
320	100	n.a.
560	100	n.a.

n.a. not applicable due to 100% mortality

Based on nominal concentrations, the 14-day LC_{50} in earthworms was estimated to be 160.2 mg A-7402 A /kg (confidence interval ($p<0.05$) 145.7-176.1), equivalent to 40 mg as/kg soil.

RMS comments:

The study was well performed and reported and is considered as valid for the risk assessment.

B.9.6.2 Sublethal effects on earthworms

ACTIVE INGREDIENT

Reference:	**Nienstedt, K.M. (1999). A chronic toxicity and reproduction test exposing the earthworm *Eisenia foetida* to CGA 169374 tech. in OECD artificial soil. Springborn Laboratories Europe AG, Horn, Switzerland. Unpublished report no. 1047.064.630. (Syngenta File No 169374/1926).**
Guideline:	ISO 11268-2 (1998); BBA guideline VI, 2-2 (1994).
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number P.807002, purity 91%.
Species:	*Eisenia foetida.*
Treatments:	Stock solutions of difenoconazole, prepared in acetone, were sprayed over the artificial soil surface in test chambers to give nominal exposure concentrations of 100, 200, 300, 400 and 500 g as/ha. Benomyl was used as a toxic reference. The test was conducted in 2L plastic vessels (18 x 13.5 x 11.5 cm) with an area of approx. 212.5 cm^2. Each test vessel contained ca 550 g dw (or 663 g fw) of artificial soil.
Number of animals:	Ten worms (mean fresh weight 447 mg) were placed into each chamber. The test incorporated four replicate chambers for each exposure concentration, four replicates of a solvent control, which were sprayed with acetone, and four replicates of control treatment, which were sprayed with deionised water.
Duration:	28 days exposure + 28 days for cocoons to hatch.
Test conditions:	Test vessels were maintained at 18.5 - 24°C with a 16 hour day of 550 - 752 lux. Soil pH was 6.4 – 7.0, moisture 26 – 34% (based on dry weight), or 64 - 83% of maximum water holding capacity. The worms were fed with untreated dried cattle manure during the study.
Observations:	After 28 days, adult worms were removed from the soil for assessments of mortality and fresh weight. Cocoons were maintained in the soil for a further 28 days. Numbers of hatched worms were recorded after 28 days.
Data analysis:	Fisher Exact test for mortality, ANOVA or Kruskal-Wallis-ANOVA for weight and offspring data.

Results:

Mortality and reproduction data are shown in the table below. Exposure to difenoconazole concentrations up to 500 g as/ha did not have a significant effect on the mortality or biomass of adult earthworms after 28 days.

Similarly the number of juveniles hatched after a further 28 days was not significantly affected by exposure of the parental generation to concentrations up to 500 g as/ha difenoconazole.

Table B.9.6.2-1: Effect of difenoconazole on mortality and reproduction in earthworms.

Nominal concentration (g as/ha)	Day 28 mortality (%)	Day 28 weight (g)	Mean number offspring/adult
Solvent control	0	698±79	7.5±4.6
Control	0	690±80	5.2±1.6
100	0	701±81	6.0±4.3
200	0	692±72	4.8±2.0
400	5.0	723±73	4.8±2.6
500	2.5	705±80	4.9±2.1
Benomyl 30 mg/kg dry soil	83	n.a.	n.a.

Based on the absence of statistically significant effects on mortality, biomass or reproduction at 500 g as/ha, the NOEC for difenoconazole in earthworms was set to 500 g as/ha.

RMS comments:

No analytical measurements were conducted during the test, and therefore the test concentrations were not verified. However, this is not required in the referred guidelines. Variability of mean number of off-spring per adult in the control and solvent control was 29.9% and 61.4%, respectively. According to OECD guideline 222 for earthworm reproduction, the solvent control did not meet the validity criterium of <30% variability in the control. There was also large variation at all treatment levels (40 – 70% variability), and the 29.9% variation in the untreated control must be regarded as a borderline. Therefore, the statistical power of the ANOVA analysis (based on combined controls) is low.

In an additional submission, the notifier provided a statistical re-evaluation of the data, where the solvent control was excluded. No statistical difference in number off-spring could be detected using Mann-Whitney U-test and Dunnett's test. However, this is not surprising in the light of the large variation in all treatment groups. From the RMS point of view, this study gives no information on possible effects on earthworm reproduction. The study can not be considered as valid for the risk assessment. The risk assessment will therefore be based on studies with the relevant representative formulations.

METABOLITES

Only one of the major metabolites in soil (CGA 71019) was tested, although for this metabolite this was not required according to the Terrestrial Guidance Document, since the DT_{90} in soil was <100 days. For CGA 205375, the trigger of DT_{90}>100 days was exceeded, and based on the acute tests on earthworms this metabolite was shown to be more toxic than the parent compound. Therefore, the long term toxicity needs to be addressed. A study is ongoing, and the final report will be submitted in July 2006, to be included in an Addendum to this DAR.

Reference: **Ehlers HA (2000). Effects of 1,2,4-triazole on reproduction and growth of earthworms *Eisenia fetida* (Savigny 1826) in artificial soil. IBACON GmbH,**

	Rossdorf, Germany. Unpublished report no. 7781022. (Syngenta File No 64250/4385)
Guideline:	ISO 11268-2 (1998); BBA guideline VI, 2-2 (1994)
GLP:	Yes.

Material and methods:

Test substance:	Technical CGA 71019; Batch no. R 200; Purity 99%.
Species:	Earthworms (*Eisenia fetida andrei*).
Treatments:	CGA 71019 was mixed into artificial soil (OECD) to give nominal exposure concentrations of 7.08, 35.41 and 70.81 µg/kg soil corresponding to application rates of 5.31, 26.56 and 53.11 g CGA 71019/ha.
Number of animals:	The test incorporated four replicate chambers for each exposure concentration, four replicates of a control, in which soil was treated with deionised water, and four replicates of toxic standard, in which soil was treated with carbendazim at 2.624 mg as/kg. Ten worms (weight 351-550 mg) were placed into each chamber.
Duration:	A four-week exposure phase was followed by a four-week reproduction phase.
Test conditions:	Test vessels were maintained at 20 ± 2°C with a 16 hour day of 400-700 Lux. Soil pH was 5.7 – 5.8, and moisture content was 46 - 54% of the maximum water holding capacity. Finely ground cattle manure was mixed into the soil at start, and once per week for the first 3 weeks of the test.
Observations:	After 28 days, adult worms were removed from the soil for assessments of mortality and fresh weight. Cocoons were maintained in the soil for a further 28 days. Numbers of hatched worms were recorded after 28 days.
Data analysis:	Dunnett test and Bonferroni Holm-test.

Results:

After 28 days, there was no mortality in the control, CGA 71019 and toxic standard treatment groups.

Reproduction data are shown in the table below. Exposure to the toxic standard, carbendazim, significantly ($p<0.05$) reduced reproduction rates compared to the controls.

Exposure to CGA 71019 concentrations up to 70.81 µg/kg soil (the highest rate tested) did not have a significant effect on the mortality, growth or feeding activity of earthworms. Similarly the number of juveniles hatched after a further 28 days was not significantly affected by exposure of the parental generation to concentrations up to 70.81 µg CGA 71019/kg soil.

Table B.9.6.2-2: Effect of CGA 71019 on earthworm weight and reproduction

Nominal concentration (µg/kg)	Day 28 weight (mg/worm)	Mean number offspring/adult
Control	108	172
7.08	134	148
35.41	129	168
70.81	120	176
Toxic standard	96	6*

**significantly different from control value ($p \leq 0.05$)*

Based on mortality, growth, reproduction and feeding activity the 56-day NOEC of 1,2,4-triazole for *Eisenia fetida* was 0.0708 mg /kg soil, the highest concentration tested.

RMS comments:

The study was generally well performed and reported. No analytical measurements were conducted during the test, and therefore the test concentrations were not verified. However, this is not required in the referred guidelines, and since the test compound is not expected to be rapidly degraded or volatilised, the test is considered to be valid.

FORMULATED PRODUCTS

DIVIDEND 030FS

Reference:	**Friedrich, S. (2002). Sublethal toxicity of CGA 169374 FS 030 (A-9142 G) to the earthworm *Eisenia fetida*. BioChem Agrar, Gerichshain, Germany. Unpublished report no. 02 10 48 036. Study dates 7 June – 2 August 2002 (Syngenta File No. CGA 169374/2242)**
Guideline:	ISO 11268-2 (1998).
GLP:	Yes.
Material and methods:	
Test substance:	A-9142 G containing 30.1 g/L CGA 169374. Batch no. P.902001.
Species:	Earthworms (*Eisenia foetida*).
Treatments:	Worms were exposed to A-9142 G mixed into artificial soil to give nominal concentrations of 0.02, 0.5, 0.1, 0.2 and 0.4 mg as/kg dry soil.
Number of animals:	The test incorporated four replicate test chambers of 10 worms for each exposure concentration, four replicates of an untreated control prepared with deionised water, and four replicates of a toxic standard in which soil benomyl (Benlate) was mixed into soil at 0.833 mg as/kg dry soil.
Duration:	56 days exposure.
Test conditions:	Chambers were maintained in a controlled environment at 20±2°C with a 16 hour day of 400-800 Lux. Soil pH was 6.0±0.5, and moisture 40 - 60% of maximum water holding capacity. Finely ground cattle manure was mixed into the soil at start, and once per week for the first 4 weeks of the test.
Observations:	Adult earthworms were removed from the soil on day 28 for assessment of mortality, health and weight. On day 56, test vessels were emptied in order to count numbers of juvenile worms.
Data analysis:	Dunnett test and Williams-test. ANOVA.

Results:

Mortality, bodyweight and fecundity data are presented in the table below. Exposure to benomyl did not cause any mortalities during the first 28 days of exposure but significantly reduced biomass increase by 18% and caused a significant 68% reduction in the number of juveniles produced per replicate. Exposure to A-9142 G concentrations did not have significant adverse effects on earthworm mortality or weight increases up to 28 days. Similarly, concentrations up to 0.2 mg as/kg dry soil did not have a significant effect on reproduction. However, the number of juveniles produced per replicate was reduced by 40% following exposure to 0.4 mg as/kg dry soil.

Table B.9.6.2-3: Effect of A-9142 G on earthworm mortality, weight and reproduction.

Nominal concentration (mg as/kg)	Mortality (%)	Mean weight at study initiation (mg)	Mean weight after 28 days (mg)	Mean weight increase by Day 28 (%)	Mean offspring per replicate
Control	0	414.3±32.9	512.0±84.1	23.5	220.0
0.02	0	413.2±30.5	558.3±91.4	35.1	263.5
0.05	0	417.7±31.5	534.5±112.4	27.9	209.0
0.1	0	415.4±37.1	560.5±100.7	35.1	202.5
0.2	0	413.0±29.6	567.4±107.6	37.2	212.0
0.4	0	420.1±34.7	561.6±105.7	33.5	131.3*
Benomyl (0.833)	0	415.9±33.9	438.2±54.3	5.5*	70.8*

** significantly different from control ($p \leq 0.05$)*

Contact exposure to A-9142 G applied to soil at rates up to 0.4 mg as/kg dry soil did not have any significant adverse effects on earthworm mortality or body weight after 28 days. Based on the absence of any significant effects on the number of juveniles present after 56 days, the NOEC was considered to be 0.2 mg as/kg.

RMS comments:

The study was generally well performed and reported. There seemed to be an increase in body weight among treated worms after 28 days compared to the control. However, the effect was not statistically significant and the effect did not seem to be dose related. Therefore, the RMS agrees with the proposed NOEC value of 0.2 mg as/kg. This value will be used in the risk assessment of the representative seed treatment use of difenoconazole.

Reference:	**Nienstedt, K.M. (2000). A chronic toxicity and reproduction test exposing the earthworm *Eisenia fetida* to CGA 169374 FS 030 (A-9142 G) treated wheat seeds in OECD artificial soil. Springborn Laboratories AG, Horn, Switzerland. Unpublished report no. 1047.047.630. Study dates 4 May – 30 June 1999 (Syngenta File No. CGA 169374/1991)**
Guideline:	ISO 11268-2 (1998); BBA Guideline VI, 2-2 (1994).
GLP:	Yes.
Material and methods:	
Test substance:	Wheat seeds (Runal) treated with A-9142 G at 60 mg/kg CGA 169374. Seed lot number ST 15 TA 576.
Species:	Earthworm (*Eisenia fetida).*
Treatments:	Adult earthworms with a mean weight of 450 mg were introduced into test vessels containing 3 kg artificial soil and wheat seeds, treated with A-9142 G at a rate of 60 mg CGA 169374/kg seed. Seeds were buried 1-2 cm deep to give a sowing density of 300 kg seeds/ha.
Number of animals:	The test incorporated four replicate chambers of twenty worms for the test substance treatment and four replicates of an untreated control in which seeds were untreated.
Duration:	28 days exposure of adults, 28 days for juveniles.
Test conditions:	Chambers were maintained in a controlled environment at 20±2°C with a 16 hour day of 2000 Lux. Soil pH 5.9, moisture 60% of maximum water holding capacity. Worms were fed daily with dried cattle manure during the test.
Observations:	Adult earthworms were removed from the substrate on day 28 for assessment of

	mortality, health and weight. On day 56, test vessels were placed in water baths at 50-60°C to encourage juveniles to the soil surface for counting.
Data analysis:	Yates corrected Chi-square test and t-test.

Results:

Mortality and fecundity data are presented in the table below. Exposure to A-9142 G did not have significant adverse effects on earthworm mortality recorded on day 28 or offspring numbers recorded on day 56. Instead, exposure to A-9142 G caused a significant 51% increase in day 28 bodyweight relative to untreated worms.

Table B.9.6.2-4: Effect of A-9142 G on earthworm mortality, weight and reproduction. Exposure to wheat seed treated with A-9142 G at a rate of 60 mg CGA 169174/kg seed and a sowing density of 300 kg/ha (equivalent to 18 g as/ha).

Treatment	Mortality (%)	Mean weight increase by Day 28 (mg)	Mean offspring per adult
Control	0	183	15.3
A-9142 G	1.3	277*	13.4

** significantly different from control ($p \leq 0.05$)*

Exposure to wheat seed treated with A-9142 G at a rate of 60 mg CGA 169174/kg seed and a sowing density of 300 kg/ha (equivalent to 18 g as/ha), did not have any significant effect on earthworm mortality or number of off-spring per adult, but resulted in an increased body weight of the worms compared to the control.

RMS comments:

The study was generally well performed and reported. The observed increase in body weight gain among treated worms compared to the control (51% effect) was considered as not adverse, since it was not coupled with a decreased number of off-spring. In a review of endpoints from earthworm reproduction tests (**Kula, 1998**)[16], referred to by the notifier, this conclusion was supported although it was stated that "there is a lack of knowledge about the importance of increased weight gain and about whether and how this parameter is relevant for field populations". In this case, there is also a second study (Friedrich, 2002) on the same formulation, where no statistically significant effects on body weight gain were observed at similar dose levels. Therefore, the RMS can agree that the observed effect could be of less importance. The results from this study will not be used in the risk assessment.

SCORE 250EC

Reference:	**Nienstedt, K.M. (2001). A chronic toxicity and reproduction test exposing the earthworm *Eisenia fetida* to CGA 169374 250 EC (A-7402 G). Springborn Laboratories AG, Horn, Switzerland. Unpublished report number 1047.100.631. (Syngenta No. CGA 169374/2140). 23 November – 23 January 2001.**
Guideline:	ISO 11268-2 (1998); BBA Guideline VI, 2-2 (1994).
GLP:	Yes.

Material and methods:

[16] *Kula, C (1997). Endpoints in laboratory testing with earthworms; experience with regard to regulatory decisions for plant protection products. In Advances in Earthworm Ecotoxicology. Sheppard, S* et al. *(eds). Proceedings from the second international workshop on earthworm ecotoxicology. 2-5 april, 1997. Amsterdam, the Netherlands. SETAC Technical Publication Series.*

Test substance:	A-7402 G containing 263 g CGA 169374/L. Batch number WM 002163.
Species:	Earthworm (*Eisenia fetida).*
Treatments:	Adult earthworms with a mean weight of 448 mg were introduced into test vessels containing 550 g artificial soil mixed with 5.5 g cow manure and deionised water. After earthworms had burrowed into the test medium, solutions of A-7402 G prepared in deionised water were sprayed over the soil surface using a laboratory sprayer delivering 600 L/ha to give nominal concentrations of 125, 625, 1250 and 2500 g as/ha.
Number of animals:	The test incorporated 4 replicate test vessels of 10 worms for each exposure concentration and 6 replicate vessels of 10 worms for a control treatment, prepared by spraying with water.
Duration:	28 days exposure of adults, 28 days for juveniles.
Test conditions:	Chambers were maintained in a controlled environment at 20±2°C with a 16 hour day of 400 - 800 Lux. Soil pH 6.2, moisture ca 50%. Worms were fed weekly with dried cattle manure during the test.
Observations:	Adult earthworms were removed from substrate on day 28 for assessment of mortality, health and weight. On day 56, test vessels were placed in water baths at 50-60°C to encourage juveniles to the soil surface for counting.
Data analysis:	Fisher Exact test, ANOVA, Dunnetts test.

Results:

Mortality, weight and reproduction data are presented in the table below. Data indicates that exposure to A-7402 G did not have significant effects on earthworm mortality recorded on day 28. Exposure to 125, 1250 and 2500 g as/ha significantly increased the earthworm weight by 43, 43 and 66%, respectively, relative to control worms. Earthworm reproduction was not affected following exposure to A-7402 G at concentrations of 125, 625 or 1250 g as/ha. However, exposure to 2500 g as/ha significantly reduced the number of juveniles produced by 41% relative to untreated worms.

Table B.9.6.2-5: Effect of A-7402 G on earthworm mortality, weight and reproduction

Nominal concentration (g as/ha)	Mortality (%)	Lesions (%)	Mean weight at study initiation (mg)	Mean weight after 28 days (mg)	Mean weight increase by Day 28 (mg)	Mean juvenile number per vessel
0	1.7	3.3	450±26	682±21	231±25	479.3±138.6
125	0	2.5	443±26	773±83	330±72*	602.8±92.4
625	2.5	0	445±26	735±25	290±21	482.3±102.1
1250	0	0	445±28	775±44	330±46*	449.3±128.1
2500	0	0	456±28	806±17	350±19*	282.3±39.8*

** significantly different from control (p≤0.05)*

Based on earthworm mortality and lesions, the 28-day NOEC was estimated to be 2500 g as/ha. The NOEC based on reproduction was considered to be 1250 g as/ha. Using standard assumptions of soil bulk density of 1.5 g/cm^3 and an incorporation depth of 5 cm, the NOEC for reproduction effects is equivalent to 1.7 mg as/kg.

RMS comments:

Based on reproductive effects, the RMS agrees with the NOEC value proposed by the notifier. However, based on the significant effects on weight gain also at the lowest treatment level, no NOEC could be determined from this study (<0.17 mg as/kg soil). The observed increase in body weight gain among treated worms compared to the control was considered as not adverse, since it was not coupled with a decreased number of off-spring. In a review of endpoints from earthworm reproduction tests (**Kula, 1998**)[17], referred to by the notifier, this conclusion was supported although it was stated that "there is a lack of knowledge about the importance of increased weight gain and about whether and how this parameter is relevant for field populations". In this case, the notifier also referred to two studies (**Friedrich, 2003 and Friedrich, 2005,** not evaluated in detail) on other suspension concentrate formulations containing difenoconazole and azoxystrobin or paclobutrazole (two active ingredients with low toxicity to earthworms), where no statistically significant effects on body weight gain were observed at similar dose levels of difenoconazole (overall NOEC based on the content of difenoconazole was 3.3 – 4.5 mg/kg dw and no treatment related effects were seen on body weight change. Based on this additional information, the RMS considers that the observed effect on body wheight could be of less importance in field populations of earthworms, and agrees with the proposed NOEC for reproductive effects for this formulation. However, the acceptability of this approach may need further discussion.

B.9.6.3 Bioconcentration in earthworms

Reference:	**van der Kolk, J. (2001). A bioconcentration test exposing the earthworm *Eisenia foetida* to ^{14}C-CGA 169374 in OECD artificial soil. Springborn Laboratories Europe AG, Switzerland. Unpublished report no. 1047.103.632. (Syngenta File No 169374/2141)**
Guideline:	ISO 11268-2 (1998); BBA guideline VI, 2-2 (1994); OECD 305 (1996).
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number AMS 25/3, purity 99.3%) ^{14}C-difenoconazole, batch number Cl-XLVI-21, purity 92%.
Species:	Earthworms (*Eisenia foetida*).
Treatments:	Worms were exposed to ^{14}C-difenoconazole, prepared in acetone and mixed with artificial soil to give a nominal concentration of 1.55 mg/kg soil. After a 56-day uptake phase, worms were transferred to untreated soil for an 83-day depuration phase.
Number of animals:	The test incorporated 52 replicate treated vessels of 10 worms and 6 replicate vessels of 10 worms for each of two control treatments, prepared with and without acetone.
Duration:	56 days uptake phase.
Test conditions:	Test vessels were maintained at 20±2°C with a 16 hour day of 419-798 Lux. Soil moisture content was 60% of maximum water holding capacity, and soil pH was 6.4

[17] *Kula, C (1997). Endpoints in laboratory testing with earthworms; experience with regard to regulatory decisions for plant protection products. In Advances in Earthworm Ecotoxicology. Sheppard, S* et al. *(eds). Proceedings from the second international workshop on earthworm ecotoxicology. 2-5 april, 1997. Amsterdam, the Netherlands. SETAC Technical Publication Series.*

– 6.5. Earthworms were fed with dried cattle manure on days 4, 13, 21, 35, 63, 78, 92, 113 and 127.

Observations: Worms were removed from 4 replicate treated vessels on 12 occasions (days 4, 7, 12, 21, 27, 40, 56, 63, 70, 84, 105, 139) for measurement of total radioactivity by combustion followed by liquid scintillation counting. The radioactive content of soil from vessels sampled on days 4, 7, 12, 21, 40 and 56 was determined by the same method. Soil samples collected on days 4, 7 and 56 and worm samples from days 4, 7, 56 and 139 were extracted in methanol for identification of 14C-CGA 169374 content by HPLC. In addition, earthworm mortality, general health and weight were recorded for 4 replicate control, solvent control and treated vessels on day 28.

Results:

Uptake and depuration data are presented in the table below. Data indicates (not presented) that exposure to difenoconazole did not have significant adverse effects on earthworm mortality, behaviour or weight recorded on day 28. During the 56-day uptake phase, the concentration of difenoconazole in soil remained constant at 1.52 mg/kg while there was a gradual accumulation of total radioactivity in earthworm tissues up to a maximum of 1.24 mg/kg on day 56, excluding gut contents. A steady state was not reached during the uptake phase, the concentration found in worms remained below that recorded in soil and therefore the BCF for day 56 was <1. After transfer to untreated soil, the concentrations of ^{14}C-difenoconazole found in worms declined to 40% of day 56 concentrations by day 84. HPLC analyses indicated that ^{14}C-difenoconazole accounted for 86.7% of total radioactivity in all soil extracts. TLC analyses of worm extracts showed that the proportion of radiolabel existing as ^{14}C-difenoconazole, declined from 31.4% on day 4 to below detection limits by day 56.

Table B.9.6.3-1: Uptake and depuration of difenoconazole in earthworms.

Phase	Day after application	^{14}C concentration in soil (mg/kg)	^{14}C concentration in worms* (mg/kg)	Bioconcentration factor
Uptake	4	1.57	1.15	0.73
	7	1.60	0.51	0.31
	12	1.52	0.59	0.39
	21	1.46	0.66	0.45
	27	1.59	0.73	0.46
	40	1.48	1.00	0.68
	56	1.43	1.24	0.87
Depuration	63	n.d.	0.71	n.a.
	70	n.d.	0.58	n.a.
	84	n.d.	0.49	n.a.
	105	n.d.	0.59	n.a.
	139	n.d.	0.52	n.a.

**excluding gut contents*
n.d. not determined
n.a. not applicable

RMS comments:

For the risk assessment of secondary poisoning of birds and mammals, the whole worm BCF should be used. Since the gut content of radioactive residues was not included in the analysis of this study, and steady state was not reached, the BCF value was probably under-estimated. Further, although in accordance with the referrd

guidelines, feeding the worms with manure that was not treated with difenoconazole would also have contributed to a lower overall exposure. Therefore, a calculated value based on the log P_{ow} will be used as a worst case for the risk assessment.

B.9.6.4 Summary and risk assessment for earthworms

A summary of available studies on earthworms is given in the table below.

Table B.9.6.4-1: Summary of earthworm toxicity endpoints for difenoconazole, the soil metabolites CGA 71019 and CGA 205375, and the representative formulations DIVIDEND 030FS and SCORE 250EC.

Test substance	Endpoint	Value (mg as/kg soil)	Reference
SHORT TERM			
Difenoconazole	14-day LC_{50}	>610	Surprenant (1987c)
CGA 71019	14-day LC_{50}	>1000	Heimbach (1986)
CGA 205375	14-day LC_{50}	312 (284 – 343)	Batscher (2002)
DIVIDEND 030FS	no short term data		
SCORE 250EC	14-day LC_{50}	40 (36 – 44)	Thun (1993)
LONG TERM			
Difenoconazole	no reliable data		Nienstedt (1999c)
CGA 71019	28-day NOEC	0.0708 (reproduction)	Ehlers (2000)
CGA 205375	no long term data	ongoing study	
DIVIDEND 030FS	56-day NOEC	0.2 (reproduction)	Friedrich (2002)
SCORE 250EC	56-day NOEC	1.7 (reproduction)	Nienstedt (2001)

**this value was not fully reliable, and should be treated with caution. The formulation data will be used for the risk assessment. However, the study is considered to fulfil the data requirement.*

Only one of the major metabolites in soil (CGA 71019) was tested for effects on reproduction, although for this metabolite this was not required since the DT_{90} in soil was <100 days. For CGA 205375, the trigger of DT_{90}>100 days was exceeded, and based on the acute tests on earthworms this metabolite was shown to be more toxic than the parent compound. Therefore, the long term toxicity needs to be addressed. A study is ongoing, and the final report will be submitted in July 2006, and will be evaluated in an Addendum to this DAR.

Further, the available long term study on the active ingredient was not considered as reliable due to a very high variability in control and treated groups. Based on recommendations in the Guidance Document On Terrestrial Ecotoxicology (SANCO/10329/2002), certain study types (for example non-target arthropod studies, the earthworm reproduction test and the soil micro-flora test) may be conducted with a formulated product instead of the active substance. Hence, it is considered appropriate to use the more reliable NOEC from available formulation studies (0.2 mg as/kg dw for DIVIDEND 030FS and 1.7 mg as/kg dw for SCORE 250EC) for the risk assessment as proposed by the notifier.

It should be kept in mind that since the worms were fed with untreated cattle manure during the tests, it could be argued that only the contact route of exposure was included, and the total exposure levels were probably under-estimated compared to field conditions. However, this is a normal procedure for this type of test, and was also in accordance with the recommendations in the referred guidelines.

B.9.6.4.1 SEED TREATMENT WITH DIVIDEND 030FS

Calculations of the predicted soil concentrations of difenoconazole and its metabolites, CGA 71019 and CGA 205375 at the use of the representative seed treatment formulation, are described in **Document MIII Section 5,** and evaluated by the RMS in Annex B.8. The resulting PEC_s values are presented in the table below.

Table B.9.6.4-2: Soil PEC values for difenoconazole and metabolites at the representative use of the seed treatment formulation DIVIDEND 030FS.

Test substance	Soil PEC (mg as/kg)
Difenoconazole	0.016
CGA 71019	0.0006
CGA 205375	0.0014

In order to evaluate the potential risk of difenoconazole and its soil metabolites to earthworms following the proposed uses of DIVIDEND 030FS, acute and long term toxicity exposure ratios (TER_A and TER_{LT}) have been calculated considering the toxicity data and the maximum predicted environmental concentrations in soil (PEC_S). As the log P for difenoconazole and CGA 205375 are greater than 2 (log P_{ow} = 4.4 and 3.8, respectively), the 14 day LC_{50} and NOEC values were reduced by a factor of 2 to account for the relatively high organic matter content of the artificial test soil compared to agricultural soils. Log P_{ow} values for the soil metabolite CGA 71019 is less than 2 and does not require adjustment. The resulting TER values are presented in the table below.

Table B.9.6.4-3: Short and long-term TER values for difenoconazole in earthworms at the representative use of the seed treatment formulation DIVIDEND 030FS. Risk assessment based on formulation study.

Test substance	Soil PEC (mg as/kg)	Short term		Long-term	
		14-day LC_{50} (mg/kg soil)	TER	56-day NOEC (mg/kg soil)	TER
Difenoconazole	0.016	>610	>19000	no reliable data	-
DIVIDEND 030FS	0.016	>0.4	13	0.2	6.3
CGA 71019	0.0006	>1000	>770000	0.0708	54
CGA 205375	0.0014	156	111000	n.d	n.d.

n.d. not determined

The TER values for earthworms exceeded the short and long-term Annex VI triggers of 10 and 5, respectively, indicating that no further refinement is needed.

RMS comments:

The RMS generally agrees with the risk assessment provided by the notifier. No long term data was available for the metabolite CGA 205375, but a study is ongoing and will be included in an Addendum of the DAR.

B.9.6.4.2 SPRAY APPLICATIONS WITH SCORE 250EC

Maximum predicted soil concentrations of difenoconazole and the metabolites, CGA 71019 and CGA 205375, following applications of SCORE 250EC according to use patterns in pome fruit and carrots were calculated by the notifier as described in Document M-III Section 5, and evaluated by the RMS in Annex B.8. Difenoconazole PECs were estimated assuming 65 and 80% foliar interception in pome fruit and carrots, respectively, and a soil DT_{50} of 246 days (RMS value). Values for CGA 71019 and CGA 205375 were estimated assuming zero

degradation and 65 and 80% foliar interception in pome fruit and carrots, respectively. The resulting values are presented in the table below.

Table B.9.6.4-4: Soil PEC values for difenoconazole, CGA 71019 and CGA 205375 at the representative use of the spray formulation SCORE 250EC. Based on RMS corrected values.

Crop	Application rate (g as/ha)	Number of applications	Application interval (days)	Soil PEC (mg as/kg)		
				Difenoconazole	CGA 71019	CGA 205375
Pome fruit	75	4	7	0.136	0.005	0.012
Carrots	125	3	14	0.096	0.004	0.008

The potential short term risk of difenoconazole to earthworms was evaluated by calculation of a toxicity exposure ratio (TER) between soil PEC and the 14-day LC_{50}. For compounds with a log P_{OW} value greater than 2, the LC_{50} is reduced by a factor of 2 in order to account for the relatively high organic matter content of the artificial test soil compared to agricultural soils. The TER_A was calculated as follows:

$$TER_A = \frac{\text{Corrected } LC_{50} \text{ (g ai/kg)}}{PEC_s \text{ (g ai/kg)}}$$

The potential long-term risk of difenoconazole to earthworms was assessed by calculation of a long-term TER (TER_{LT}) between the maximum PEC and the 28-day reproduction NOEC as shown below. As for the acute risk assessment, the NOEC is reduced by a factor of 2 to account for the relatively high organic matter content of the test soil.

$$TER_{LT} = \frac{\text{Corrected NOEC (mg/kg)}}{PEC_s \text{ (mg/kg)}}$$

Short and long-term TER values for difenoconazole were calculated to 2 significant figures using an LC_{50} of 40 mg/kg and a 28-day NOEC of 1.7 mg/kg, respectively, based on a study with the formulation. The resulting values provided by the notifier (based a study with the representative spray formulation) exceed the relevant Annex VI triggers.

Table B.9.6.4-5: Short and long-term TER values for difenoconazole in earthworms at the representative use of the spray formulation SCORE 250EC. LC_{50} of 40 mg/kg and a 56-day NOEC of 1.7 mg as/kg from a study with the formulation was used.

Crop	Application rate (g as/ha) x number of applications	Application interval (days)	Soil PEC (mg as/kg)	Acute TER	Long-term TER
Pome fruit	75 x 4	7	0.136	147	6.3
Carrots	125 x 3	14	0.096	208	8.9

Short and long-term TER values for the difenoconazole metabolite, CGA 71019, were calculated using an LC_{50} of 1000 mg/kg and a 56-day NOEC of 0.0708 mg/kg, respectively. The resulting values exceed the relevant Annex VI triggers, indicating that CGA 71019 poses low acute risk to earthworms.

Table B.9.6.4-6: Short and long-term TER values for CGA 71019 in earthworms at the representative use of the spray formulation SCORE 250EC. Based on LC_{50} >1000 mg/kg and a 56-day NOEC of 0.0708 mg as/kg

Crop	Soil PEC (mg/kg)	Acute TER	Long-term TER
Pome fruit	0.005	190000	14
Carrots	0.004	260000	18

The acute risk of CGA 205375 was assessed from a toxicity exposure ratio between the 14-day LC_{50} of 312 mg/kg and the maximum soil PEC. The resulting acuteTER values are greater than the trigger of 10 and indicate negligible acute risk to earthworms.

Table B.9.6.5-7: Acute TER values for CGA 205375 in earthworms at the representative use of the spray formulation SCORE 250EC. Based on LC_{50} of 312 mg/kg, correction factor 2 since log Pow =3.8.

Crop	Soil PEC (mg/kg)	Acute TER
Pome fruit	0.012	13000
Carrots	0.008	19000

RMS comments and overall conclusion for earthworms:

No long term data was available for the metabolite CGA 205375, but a study is ongoing and will be included in an Addendum of the DAR.

In general, the RMS agrees with the risk assessment provided by the notifier. However, the possible impact on field populations of earthworms as a result of the increase in weight gain observed in long term laboratory studies may need further discussion.

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

In accordance with the Guidance Document On Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (SANCO/10329/2002), compounds with a soil DT_{90} of >100 days, must be evaluated for risk to other soil macro-organisms contributing to organic matter breakdown. Therefore, difenoconazole (soil DT_{90} of >100 days) and its metabolite, CGA 71019, were tested for effects on the soil macro-organism Collembola. In addition, effects on organic matter breakdown were investigated in a litter-bag study with the difenoconazole formulation SCORE 250EC (A-7402G). No data was provided for the major soil metabolite CGA 205375. A summary of the available studies is provided below.

B.9.7.1 Single species tests

ACTIVE INGREDIENT

Reference:	**Meister, A. (2002). Effects of CGA 169374 on reproduction of the collembola *Folsomia candida* in artificial soil. Institut fur Biologische Analytik, Germany. Unpublished report no. 8491016. (Syngenta File No 169374/2202)**
Guideline:	ISO 11267 Soil Quality.
GLP:	Yes.

Material and methods:

Test substance:	Technical difenoconazole, batch number P.807002, purity 91%.

Species:	Collembola, *Folsomia candida*
Treatments:	Technical difenoconazole was prepared in acetone, and mixed with artificial soil to produce nominal concentrations of 31.25, 62.5, 125, 250, 500 and 1000 mg/kg dw soil.
Number of animals:	The test incorporated five replicate vessels of 10 collembola for each exposure concentration, five replicates of solvent control prepared with acetone, five replicates of an untreated control and five replicates of toxic standard, in which soil was treated with phenmedipham (Betosip) at 33.1 mg as/kg dw soil.
Duration:	28 days.
Test conditions:	Temperature 19 – 21°C, light intensity 500 – 800 Lux (16 hours per day). Soil pH was 7.1 – 7.2 at the start, and moisture 46 – 50% of the maximum water holding capacity.
Observations:	After 28 days, the number of surviving adults and juveniles were recorded and observed for abnormalities.
Data analysis:	Fisher Exact test, Dunnett test, Mann-Whitney U-test.

Results:

Exposure to the toxic standard caused 30% mortality in adult springtails after 28 days and reduced the production of juvenile springtails by 94% compared to the control. Exposure to difenoconazole concentrations up to 500 mg/kg dw soil did not significantly affect the survival of adult springtails whereas exposure to 1000 mg/kg dw soil significantly reduced survival to 50%. Difenoconazole concentrations of 31.25 and 500 mg/kg dw did not affect reproduction while concentrations of 62.5 and 250 mg/kg dw significantly increased juvenile number and concentrations of 125 and 1000 mg/kg dw significantly reduced juvenile number. The effects on reproduction seen at 125 mg/kg dw were not considered to be treatment related.

Table B.9.7.1-1: Effects of difenoconazole on the survival and reproduction of *Folsomia candida*.

Nominal concentration (mg/kg dw soil)	Survival of adults (%)	Mean number of juveniles
Control	92	648±96
Solvent control	80	670±117
31.25	86	683±29
62.5	82	899±193*
125	80	215±115*
250	84	885±249*
500	86	650±71
1000	50*	220±138*
Toxic standard	70	42±18*

**significantly different from controls (p≤0.05)*

The 28-day NOEC for difenoconazole was considered to be 500 mg/kg dw, while the LC_{50} was estimated to be >1000 mg/kg dw based on adult survival and 894 mg/kg dw based on reproduction.

RMS comments:

The study was well performed and reported. The RMS agrees with the author of the study that the effects seen at concentrations lower than 500 mg/kg soil was probably not treatment related since at the two subsequent higher

concentrations no reduction in the number of juveniles was observed. The 28 day NOEC value of 500 mg as/kg dw soil will be used in the further assessment.

METABOLITE

Reference:	**Moser T and Scheffczyk A (2002). 1,2,4-triazole: acute and reproduction toxicity to the Collembolan species *Folsomia candida*. ECT Oekotoxikologie GmbH, Florsheim, Germany. Unpublished report no. P31CR. (Syngenta File No 71019/0053)**
Guideline:	ISO Guideline 11267 (1999).
GLP:	Yes.
Material and methods:	
Test substance:	Technical CGA 71019; Batch no NLL7052-1; Purity 99%.
Species:	Collembola, *Folsomia candida.*
Treatments:	Stock solutions of CGA 71019, prepared in deionised water, were mixed with artificial soil to produce nominal concentrations of 1, 1.8, 3.2, 5.6 and 10 mg/kg dw soil.
Number of animals:	The test incorporated five replicate vessels of 10 collembola for each exposure concentration, five replicates of a control prepared with water and five replicates treated with a toxic standard, phenmedipham (Betanal, at 23.85 mg as/kg soil).
Duration:	28 days.
Test conditions:	Temperature 20±2°C, constant light. The soil pH was 6±0.5 and moisture content was 40 – 60% of maximum water holding capacity of the soil.
Observations:	After 28 days, the number of surviving adults and juveniles were recorded and observed for abnormalities.
Data analysis:	Cochrans test, Dunnetts test, ANOVA.

Results:

Average mortality and number of juveniles in the treatment groups are given in the table below. Exposure to the toxic standard caused a significant 25% reduction in numbers of juvenile springtails relative to control, verifying the sensitivity of the test system. Exposure to CGA 71019 at concentrations up to 10 mg/kg in soil did not significantly affect the survival of adult springtails, whereas exposure at concentrations of 3.2 mg/kg soil, or more, significantly reduced juvenile springtail numbers by up to 49.8% of control values.

Table B.9.7.1-2: Effect of CGA 71019 on mortality and reproduction in *Folsmia candida*.

Concentration (mg as/kg dw soil)	Mortality (%)	Mean number of juveniles	Number of Juveniles (% of control)
Control	2	710±112	100.0
1.0	4	633±154	89.2
1.8	4	612±86	86.2
3.2	14	353±48	49.8*
5.6	12	402±60	56.6*
10	6	425±75	59.8*

**significantly different from control (p≤0.05).*

Based on effects on reproduction, the 28-day NOEC for CGA 71019 in *Folsomia candida* was 1.8 mg/kg dw of soil.

RMS comments:

The study was well performed and reported, and is considered as valid for the risk assessment. The results show that this metabolite is more toxic to *Folsomia candida* than the active ingredient.

B.9.7.2 Litter bag test

SCORE 250EC

Reference:	**Meister, A. and Schwiening (2002). Effects of A-7402 G on the decomposition of organic material enclosed in litter-bags in the field. Institut fur Biologische Analytik, und Consulting IBACON GmbH., Arheilger Weg 17, Rossdorf, Germany. Unpublished report number 8381081. (Syngenta No. CGA 169374/2228). 23 May – 21 November 2000.**
Guideline:	Draft method: litter-bag test on decomposition February 2000.
GLP:	Yes.
Material and methods:	
Test substance:	A-7402 G containing 263 g CGA 169374/ha. Batch number WM 002163.
Treatments:	A field that had been under grass for 4 years was divided into 12 plots (40 x 10 m) with a 4 m buffer between each plot. Forty litter bags (10 x 15 cm) were filled with 3 g untreated barley straw, and laid on the surface in each plot. A-7402 G (0.506 kg as/ha, ie. higher than the maximum annual dose rate of 3x125 g as/ha) was applied at a water volume of 400 L/ha using a plot sprayer operating at 3.1 bar. Separate plots were sprayed with a toxic standard, benomyl, at a rate of 4 kg as/ha. The study incorporated 4 replicate plots for each treatment and a control, which was not sprayed. One day after application, the bags were buried horizontally at a maximum depth of 5 cm.
Duration:	Approximately 6 months.
Test conditions:	Field conditions in Darmstadt-Dieburg, Germany. Soil temperature was 8 - 19°C. The soil was a loamy sand to silty sand with an organic carbon content of 0.99% and a pH of 5.4 – 5.9. The maximum water holding capacity was 42% and the soil moisture during the study was 8.5 – 21%
Observations:	After 5, 11, 15 and 24 weeks, bags were sampled for measurements of straw decomposition. For this purpose, 8 bags were removed from each plot and dried at 60 or 105°C for 24 hours before removing soil and root particles. The dry weight of the remaining straw was recorded before and after ignition at 600°C for 30 minutes. Reference samples of soil and straw were also ignited to allow determination of straw mineral content and soil organic content. After correcting for non-combusted organic matter and combusted minerals, the weight change of ash-free organic material was used for calculation of the decomposition rate.
Data analysis:	Mann-Whitney U-test.

Results:

Mean straw decomposition data is presented in the table below. Mean decomposition in litter-bags from control plots was 30.9, 48.2, 56.5 and 75.5% after 33, 76, 107 and 168 days, respectively. Levels of decomposition in plots exposed to the toxic standard were 0.9-4.9% lower than control values. These differences were significant on first, second and fourth, but not third, sampling occasions. Levels of decomposition in plots exposed to 506 g as/ha were lower than control values although these differences were only significant on first and fourth sampling occasions, when reductions of 3.6 and 17%, respectively, were recorded. Although statistically significant, the study author did not consider these observations to be biologically significant, given that decomposition in all plots was generally high. In the case of A-7402 G-treated plots, decomposition was 62.7% after 168 days.

Table B.9.7.2-1: Effect of A-7402 G on litter-bag decomposition

Sampling date (days after application)	Treatment	Mean decomposition (%)	Decomposition per day (%)
26.06.2000	Control	30.9	0.94
(33)	Toxic standard	26.0*	0.79
	A-7402 G	26.7*	0.81
08.08.2000	Control	48.2	0.40
(76)	Toxic standard	44.4*	0.43
	A-7402 G	46.7	0.46
08.09.2000	Control	56.5	0.27
(107)	Toxic standard	55.6	0.36
	A-7402 G	52.3	0.18
08.11.2000	Control	75.5	0.32
(168)	Toxic standard	66.9*	0.19
	A-7402 G	62.7*	0.17

** significantly different from control (p<0.05)*

RMS comments:

According to draft OECD guidelines for litter bag tests, the test substance should be incorporated into the soil. In this case, the bags were placed on the soil surface before application, and thereafter buried into the soil. Although the concentration in the soil profile is probably lower compared to when the substance is incorporated, this procedure can be regarded as a worst case since the litter bags were directly exposed via overspray and at a rate higher than the representative maximum annual application rate of 3x125 g as/ha.

The lack of adverse effects also in the toxic standard treatment makes the validity of the test questionable. However, this is not unusual in this type of tests, since the toxic reference (benomyl) was selected based on its toxicity to earthworms and therefore the study is accepted.

B.9.7.3 Summary and risk assessment for soil macro-organisms

A summary of the available toxicity data on other soil macro-organisms is given in the table below. Only one of the major metabolites in soil (CGA 71019) was tested, although for this metabolite this was not required since the DT_{90} in soil was <100 days. For CGA 205375, the trigger of DT_{90}>100 days was exceeded, and the long term

toxicity needs to be addressed. A study on Collembola is ongoing - the final report will be submitted in July 2006, and will be evaluated in an Addendum to this DAR.

Table B.9.7.3-1: Summary of toxicity to other soil macro-organisms for difenoconazole, the soil metabolites CGA 71019 and CGA 205375, and the representative formulations DIVIDEND 030FS and SCORE 250EC.

Test substance	Test organism	Endpoint	Value (mg as/kg dw soil)	Reference
Single species test				
Difenoconazole	*Folsomia candida*	28 day NOEC	500	Meister (2002)
CGA 71019	*Folsomia candida*	28 day NOEC	1.8	Moser and Scheffczyk (2002)
CGA 205375	No data available			
Litter bag test				
SCORE 250EC, 0.506 kg as/ha direct overspray	"Decomposers"	Reduction in decomposition rate after 168 d.	17% effect compared to control	Meister and Schwiening (2002)
DIVIDEND 030FS	No data available			

Table B.9.7.3-2: Risk assessment for other soil macro-organisms exposed to difenoconazole.

Crop	Application rate (g as/ha)	NOEC (mg as/kg soil)	PEC_{soil} (mg as/kg soil)	TER	Annex VI trigger
ACTIVE INGREDIENT					
Pome fruit	75 x 4	500	0.136	3700	10
Carrots	125 x 3	500	0.096	5200	10
Seed treatment	60 mg as/kg seed	500	0.016	31200	
METABOLITE CGA 71019					
Pome fruit	75 x 4	1.8	0.005	360	10
Carrots	125 x 3	1.8	0.004	450	10
Seed treatment	60 mg as/kg seed	1.8	0.0006	3000	10
METABOLITE CGA 205375					
		no data available	-	-	10

Since the DT_{90} value of difenoconazole was >365 days, further data was needed to address the possible effects on organic matter decomposition in soil under field conditions. In a litter bag test with the SCORE 250EC formulation applied at a rate corresponding to 0.506 kg as/ha, with direct overspray of the litter bags before burrowing into the soil, a 17% reduction in decomposition was observed after 168 days compared to the control. In the report from the EPFES workshop, an effect of 10 – 25% was proposed as trigger. The notifier argued that the EPFES workshop was held after the field phase of this study had been completed and published after the study had been reported, and that prior to the EPFES workshop, the guidance (edited version issued as BBA, 2001[18]) stated that expert judgement was needed in cases where reduction in decomposition compared to the control was between 15 and 30%. In this case the effects were within this interval (max 17% effect), and therefore the ecological significance of the effects may need to be further discussed. It should be kept in mind however, that the exposure situation in the available study was probably more "worst case" (higher dose and litter bags directly exposed) compared to the representative use of difenoconazole in carrots, pome fruit and as a seed treatment. The notifier has announced that a new litterbag study is planned to start in 2006, in order to better reflect realistic exposure conditions and the present state of the art of the available guidelines.

[18] *BBA, 2001. Minutes of a meeting on the requirement of data according to Council Directive 91/414/EEC, Annex III, point 10.6.2, organised by the BBA (Braunschweig), 27-28 November, 2000; Minutes edited by C. Kula and S. Guske, March 2001.*

In conclusion, the available studies are not considered to completely fulfil the data requirements in Annex II and III of 91/414, since the metabolite CGA 205375 still remains to be tested on Collembola. A study is ongoing and will be submitted in July 2006 in order to complete the assessment. Based on the single species tests with difenoconazole and CGA 71019 on Collembola, the risk was concluded to be low at the representative uses of difenoconazole. However, the ecological significance of the observed effects in the litter bag study compared to more realistic exposure conditions at the representative uses of difenoconazole may need further discussions.

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

The toxicity of difenoconazole and its' metabolites, CGA 71019 and CGA 205375, to non-target soil micro-organisms was investigated in soil respiration and nitrification tests. In addition, the toxicity of technical difenoconazole was tested in an agar plate test with four soil fungi species. A summary of the studies is given below.

B.9.8.1 Laboratory studies

ACTIVE INGREDIENT

Reference:	**Ellgehausen, H. (1990). The effects of CGA 169374 on the activity of soil microbes. Ciba-Geigy Ltd, Basel, Switzerland. Unpublished report no. 89EH08. (Syngenta File No 169374/0289)**
Guideline:	Not stated.
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number P 807002, purity 91.8%.
Soil:	**Collombey loamy sand**: pH 7.3, organic carbon 1.9%, clay 2.8%, silt 14.7%, sand 83%, microbial biomass 53.6 mgC/100 g soil, maximum water holding capacity 47%. **Les Evouettes silty loam**: pH 5.4, organic carbon 1.8%, clay 11%, silt 31%, sand 58%, microbial biomass 93.4 mgC/100 g soil, maximum water holding capacity 86%.
Treatments:	For nitrogen conversion assessments, soil was either unamended or amended by lucerne meal or ammonium sulphate. Stock solutions of technical difenoconazole prepared in acetone were mixed into each soil to produce nominal concentrations of 1.67 and 16.7 mg/kg soil. Additional samples were treated with acetone for use as solvent controls.
Replicates:	Both tests incorporated sufficient vessels to allow 3 replicate vessels to be sampled for each treatment on each sampling occasion.
Duration:	28 days.
Test conditions:	After treatment, soil was moistened to 40% of water capacity with water or, for those samples used for nitrification assessments, ammonium sulphate solution. Soil

was then transferred to incubation vessels for incubation in the dark at 20±2°C.

Observations: In the respiration test, CO_2, trapped in NaOH solutions, was analysed on days 7, 14, 21 and 28 while soil samples were taken for ammonium, nitrate and nitrite analysis on days 0 and 28. In the nitrification test, soil samples were taken on days 0, 7, 14, 21 and 28 for ammonium, nitrate and nitrite analysis.

Data analysis: Not stated, not needed.

Results:

CO_2 evolution and nitrogen concentration data are presented in the tables below.

Table B.9.8.1-1: Effect of difenoconazole on respiration in unamended and Lucerne-meal amended soils.

Soil source	Lucerne meal concentration (g/100g soil)	Days after treatment	Concentration (mg as/kg soil)				
			CO_2 (mg/50 g soil)			Percentage of control	
			0	**1.67**	**16.7**	**1.67**	**16.7**
Collombey	0	7	61	61.8	65.6	101	108
		14	24.9	24.8	24.2	100	97
		21	20.3	21	20.4	103	100
		28	11.6	16.4	17.3	141	149
		Total	**117.8**	**124**	**127.5**	**105**	**108**
	0.5	7	208.4	202.9	190.2	97	91
		14	59.1	55.9	54.1	95	92
		21	52.4	59.7	50.6	114	97
		28	38.7	40	35.4	103	91
		Total	**358.6**	**358.5**	**330.3**	**100**	**92**
Les Evouettes	0	7	84.7	103.8	113	123	133
		14	32.1	37.6	37.7	117	117
		21	32.9	35.3	29.2	107	89
		28	23.8	29.2	23.9	123	100
		Total	**173.5**	**205.9**	**203.8**	**119**	**117**
	0.5	7	254	254.9	243	100	96
		14	77.4	77.9	76	101	98
		21	59.3	49.8	60.3	84	102
		28	58.4	57.1	68.3	98	117
		Total	**449.1**	**439.7**	**447.6**	**98**	**100**

Table B.9.8.1-2: Effect of difenoconazole on nitrification in Lucerne-meal amended soils.

Soil source	Nitrogen form	Days after treatment	Concentration (mg as/kg soil)				
			N (mg/50 g soil)			Percentage of control	
			0	**1.67**	**16.7**	**1.67**	**16.7**
Unamended soil							
Collombey	NH_4	0	0.038	0.038	0.038	n.a.	n.a.
		28	**0.080**	**0.098**	**0.091**	**122.5**	**113.8**
	NO_2	0	0.006	0.006	0.006	n.a.	n.a.
		28	**0.006**	**0.005**	**0.005**	**83.3**	**83.3**
	NO_3	0	2.022	2.022	2.022	n.a.	n.a.

		28	**2.760**	**4.414**	**4.304**	**159.9**	**155.9**
Les Evouettes	NH_4	0	0.082	0.082	0.082	n.a.	n.a.
		28	**0.191**	**0.168**	**0.132**	**88.0**	**69.1**
	NO_2	0	0.006	0.006	0.006	n.a.	n.a.
		28	**0.007**	**0.007**	**0.008**	**100.0**	**114.3**
	NO_3	0	4.113	4.113	4.113	n.a.	n.a.
		28	**7.956**	**9.014**	**8.909**	**113.3**	**112.0**
Lucerne-meal amended soil							
Collombey	NH_4	0	0.073	0.073	0.073	n.a.	n.a.
		28	**0.121**	**0.132**	**0.128**	**109.1**	**105.8**
	NO_2	0	0.005	0.005	0.005	n.a.	n.a.
		28	**0.007**	**0.007**	**0.007**	**100.0**	**100.0**
	NO_3	0	2.034	2.034	2.034	n.a.	n.a.
		28	**5.009**	**5.427**	**5.512**	**108.3**	**110.0**
Les Evouettes	NH_4	0	0.090	0.090	0.090	n.a.	n.a.
		28	**0.169**	**0.172**	**0.170**	**101.8**	**100.6**
	NO_2	0	0.008	0.008	0.008	n.a.	n.a.
		28	**0.011**	**0.010**	**0.010**	**90.9**	**90.9**
	NO_3	0	3.920	3.920	3.920	n.a.	n.a.
		28	**7.431**	**5.605**	**6.895**	**75.4**	**92.8**

n.a. not applicable as measurements represent triplicates from same batch of soil

Table B.9.8.1-3: Effect of difenoconazole on nitrification in ammonium sulfate amended soils.

Soil source	**Nitrogen form**	**Days after treatment**	**Concentration (mg as/kg soil)**				
			N (mg/50 g soil)			**Percentage of control**	
			0	**1.67**	**16.7**	**1.67**	**16.7**
Collombey	NH_4	0	5.08	5.24	5.18	103.1	102.0
		7	0.7	1.9	2.3	271.4	328.6
		14	0.07	0.06	0.05	85.7	71.4
		21	0.28	0.05	0.05	17.9	17.9
		28	**0.06**	**0.05**	**0.05**	**83.3**	**83.3**
	NO_2	0	<0.01	<0.01	<0.01	100.0	100.0
		7	0.02	0.17	0.2	850.0	1000.0
		14	0.01	0.01	0.01	100.0	100.0
		21	0.01	0.01	0.01	100.0	100.0
		28	**0.01**	**0.01**	**0.01**	**100.0**	**100.0**
	NO_3	0	0.68	0.7	0.7	102.9	102.9
		7	5.27	4.13	3.86	78.4	73.2
		14	6.74	6.5	6.52	96.4	96.7
		21	5.91	6.62	6.61	112.0	111.8
		28	**6.27**	**6.41**	**6.56**	**102.2**	**104.6**
Les Evouettes	NH_4	0	3.97	4.01	3.94	101.0	99.2
		7	1.9	2.3	2.4	121.1	126.3
		14	0.74	1.51	2.15	204.1	290.5
		21	0.14	0.43	0.46	307.1	328.6
		28	**0.12**	**0.16**	**0.13**	**133.3**	**108.3**
	NO_2	0	<0.01	<0.01	<0.01	100.0	100.0
		7	0.01	0.01	0.01	100.0	100.0

		14	<0.01	<0.01	<0.01	100.0	100.0
		21	0.01	0.01	0.01	100.0	100.0
		28	**<0.01**	**<0.01**	**<0.01**	**100.0**	**100.0**
	NO_3	0	0.54	0.5	0.49	92.6	90.7
		7	2.35	1.98	2.01	84.3	85.5
		14	3.58	2.89	4.07	80.7	113.7
		21	4.56	3.73	3.72	81.8	81.6
		28	**4.39**	**4.17**	**4.44**	**95.0**	**101.1**

RMS comments:

The data was reported as mean values of the three replicates for each sample, and hence there was no information on variability in the control which is needed to check the validity of the test (according to the OECD 216 and 217, variability shall not be >15% in the control replicates). For the Collombey soil, RMS does not fully agree that the effects of difenoconazole on soil respiration were less than 25% after 28 days. The notifier referred to data based on the total CO_2 production over the whole study, while the criteria are usually based solely on the day 28 data. In this case the CO_2 levels were increased by 40 – 50% compared to the control in the unamended soil by day 28. However, this could reflect a decline in the control due to a depletion of available organic carbon rather than a "real" effect of the test substance. This theory was supported by the fact that no effects were observed in the same soil when amended with Lucerne-meal. Hence, this effect was not considered to be treatment related.

It was concluded that at in the Lucerne meal-amended soil, there was >50% effect on the nitrate levels at both test concentrations after 28 days in one of the soils (Collombey). This effect on nitrate levels was not that pronounced when ammonium sulphate was used as nitrogen source.

To sum up, there seemed to be a treatment related increase in nitrification rate after 28 days in one of the soils. However, the tested concentrations were significantly higher (ca 10 and 100x) than the expected PEC in soil at the representative uses of difenoconazole (max ca 0.02 mg as/kg soil). It is considered likely that at the recommended doses, the effects on nitrogen conversion would fulfil the criteria in Annex VI of Directive 91/414, or <25% effect after 100 days.

Reference:	**Grade, R. (2000). The effect of CGA 169374 tech. On the growth of soil fungi on soil-malt extract agar plates. Novartis Crop Protection AG, Switzerland. Unpublished report no. 203636. (Syngenta File No 169374/2042)**
Guideline:	OECD proposed guidelines 216 and 217.
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number P.807002, purity 91%.
Treatments:	Stock solutions of technical difenoconazole prepared in acetone were mixed into a loamy sand soil to produce nominal concentrations of 0.05, 0.167, 0.5, 1.67, 5 and 16.7 mg/kg soil. Soil samples treated with acetone were used for solvent control

	plates. Soil and malt extract agar were transferred to agar plates and, after solidifying, plates were inoculated with agar blocks containing mycelium of 1 of 4 soil fungi (*Marasmius oraedes, Paecilomyces marquandii, Phytophtora nicotianae* and *Mucor circinelloides*).
Replicates:	The test incorporated three replicate plates of each fungus for each exposure concentration and control.
Duration:	2 - 17 days incubation.
Test conditions:	Temperature 20 or 24°C, darkness.
Observations:	Mycelial growth.
Data analysis:	Dunnett's test. Logit analysis according to Finney (1971).

Results:

Fungal growth data is presented in the table below. Exposure to difenoconazole concentrations up to 16.4 mg/kg did not have a significant effect on the mycelial growth of *Paecilomyces marquandii* or *Phytophtora nicotianae* measured on day 17. Similarly, the growth of *Marasmius oraedes* and *Mucor circinelloides* was not inhibited by concentrations up to 0.49 and 4.9 mg/kg, respectively. However, exposure to 16.4 mg/kg caused a significant 13% reduction in the growth of *Mucor circinelloides* measured on day 3, while exposure to concentrations of 1.64, 4.9 and 16.4 mg/kg caused significant reductions of 12, 19 and 24%, respectively, in the growth *Marasmius oraedes* cultures measured on day 2. By day 6, significant inhibition was only apparent in those cultures exposed to 4.9 and 16.4 mg/kg.

Table B.9.8.1-4: Effect of difenoconazole on growth of four soil fungi.

Concentration (mg/kg soil)	**Mycelial growth (cm)**				
	Marasmius oraede		***Mucor circinelloides***	***Paecilomyces marquandii***	***Phytophtora nicotianae***
	Day 2	**Day 6**	**Day 3**	**Day 17**	**Day 17**
Control	3.00	8.50	6.27	5.63	6.17
0.0049	3.07	8.50	6.23	5.67	6.33
0.16	3.17	8.50	6.17	5.40	6.87
0.49	3.10	8.50	5.97	5.73	5.83
1.64	2.63*	7.57	5.93	5.67	6.30
4.9	2.43*	7.00*	5.77	5.20	6.17
16.4	2.27*	6.17*	5.47*	5.10	6.43
NOEC (mg/kg)	0.49	1.64	4.9	16.4	16.4

**significantly different from control ($p \leq 0.05$).*

RMS comments:

The study was well performed and reported, and is considered as valid for the risk assessment.

METABOLITES

Reference:	**Völkel W (2000). The effects of CGA 71019 on soil respiration and nitrification. RCC, Itingen, Switzerland. Unpublished report no. 763367. (Syngenta File No 71019/0042)**
Guideline:	OECD draft guidelines 216 and 217; EPPO, Chapter 7, Soil microflora, Vol 24 No.1, 1994.

GLP:	Yes.

Material and methods:

Test substance:	Technical CGA 71019, batch number 301/84, purity 95.8%.
Treatments:	Soil samples (150 g, sandy-loam soil, Speyer 2.3) were dispensed into 1 litre flasks and treated with quartz sand, previously spiked with technical CGA 71019 prepared in water, to give nominal concentrations of 0.035 and 0.353 mg test item/kg dry soil, corresponding to 25 and 250 g CGA 71019/ha. Additional samples were prepared with dinoseb acetate (25 mg/kg soil, corresponding to 18.75 kg/ha) as a toxic reference substance. For nitrification experiments, samples were amended with 0.7 g lucerne meal after application.
Duration:	Flask contents were sampled for respiration and nitrification measurements after 3 hours and 7, 14 and 28 days.
Test conditions:	All soil samples were moistened to 42% of the respective maximum water holding capacity with purified water and incubated in the dark at 20°C for up to 28 days.
Observations:	For measurements of short-term respiration, three aliquots of wet soil (40g) were sampled from one flask on each occasion, amended with a glucose/talc mixture and analysed for evolved CO_2 for 12 hours. For measurements of nitrate and nitrite, three aliquots of wet soil (47g) were extracted with potassium chloride (2 M), centrifuged and filtered through filter paper. This extraction process was repeated and the combined aqueous extracts analysed for nitrate and nitrite by Flow Injection Analysis.
Data analysis:	Dixon's test, Dunnett's test.

Results:

Mean respiration rates over the study period, and nitrite and nitrate concentrations measured in the test soils over the study period are given in the tables below. Respiration rates measured in samples treated with technical CGA 71019 at 0.035 and 0.353 mg/kg did not deviate more than 8.7% compared to controls at any sampling time. In soil treated with dinoseb acetate there were reductions up to 62% in respiration rates.

Nitrite concentrations in control and CGA 71019-treated samples remained below the limits of detection for the duration of the study. Samples exposed to dinoseb acetate contained nitrite concentrations between 0.1 and 11.4 mg/kg soil. After day 0, nitrate levels decreased in both the CGA 71019-treated soils and the controls. Deviations in nitrate levels in the soils treated at either 0.035 or 0.353 mg CGA 71019/kg were 5.7% or less compared to the controls throughout the test period. Dinoseb acetate-treated soil, sampled on days 7, 14 and 28, contained up to 671% more nitrate than control samples, indicating a significant inhibitory effect on soil nitrification processes.

Table B.9.8.1-5: Effect of CGA 71019 and dinoseb acetate on short-term respiration rates in glucose-amended soil.

Treatment	Concentration (mg/ kg dry soil)	Incubation period (days)	Mean respiration rate (ml CO_2/h per kg dry soil)	Deviation from control (%)
Control	0	0	5.14	-
		7	5.03	-
		14	4.39	-
		28	4.11	-
CGA 71019	0.035	0	5.38	4.7
		7	5.32*	5.8
		14	4.72*	7.7
		28	4.34	5.5
	0.353	0	5.43*	5.7
		7	5.45*	8.2
		14	4.77*	8.7
		28	4.45*	8.3
Dinoseb acetate	25.0	0	3.10*	-39.6
		7	2.21*	-56.1
		14	1.70*	-61.1
		35	1.17*	-62.1

** significantly different from control ($p \leq 0.05$)*

Table B.9.8.1-6: Effect of CGA 71019 and dinoseb acetate on nitrite and nitrate levels in soil after amendment with lucerne meal.

Treatment	Concentration (mg/ kg dry soil)	Incubation period (days)	Nitrite		Nitrate	
			Mean NO_2^--N (mg NO_2^--N / kg dry soil)	Deviation from control (%)	Mean NO_3^--N (mg NO_3^--N / kg dry soil)	Deviation from control (%)
Control	0	0	0.1	-	8.2	
		7	<0.1	-	0.7	-
		14	<0.1	-	8.7	-
		28	<0.1	-	13.5	-
CGA 71019	0.035	0	0.1	0	7.4*	-9.8
		7	<0.1	n.a.	0.7	0.0
		14	<0.1	n.a.	8.2	-5.7
		28	<0.1	n.a.	12.8	-5.2
	0.353	0	0.1	0	7.3*	-11.0
		7	<0.1	n.a.	0.7	0.0
		14	<0.1	n.a.	8.8	1.1
		28	<0.1	n.a.	13.3	-1.5
Dinoseb acetate	25	0	0.1	0	6.8*	-17.0
		7	1.3	n.a.	5.4*	671.4
		14	11.4	n.a.	31.9*	266.7
		28	0.1	n.a.	25.3*	87.4

n.a. not applicable
** significantly different from control ($p \leq 0.05$)*

CGA 71019 at up to the highest rate of 0.353 mg/kg in soil caused less than 25% effect on respiration and nitrification processes in soil, indicating that , CGA 71019 is not expected to result in adverse effects on carbon cycles or organic matter turn-over.

RMS comments:

The study was well performed and reported, and is considered as valid for the risk assessment.

Reference: **Seyfried, B. (2002). The effects of CGA 205375 (metabolite of CGA 169374) on soil respiration and nitrification. RCC Ltd., CH4452 Itingen, Switzerland. Unpublished report no. 808176. (Syngenta File No 205375/0019)**

Guideline:	OECD 216 and 217 (January 2000); EPPO Chapter 7, Volume 24 (1) (1994).
GLP:	Yes.

Material and methods:

Test substance:	CGA 205375, batch number MLA-412/2, purity 99%.
Treatments:	Soil samples (150 g, sandy loam soil, Speyer 2.3) were dispensed into 1 litre flasks and treated with quartz sand, previously spiked with CGA 205375 prepared in acetone, to give nominal concentrations of 0, 0.09 and 0.22 mg CGA 205375/kg dry soil. Additional samples were prepared with dinoseb acetate (25 mg/kg soil) as a reference substance. For nitrification experiments, samples were amended with 0.7 g lucerne meal after application.
Duration:	Flask contents were sampled for respiration and nitrification measurements after 3 hours and 7, 14 and 28 days.
Test conditions:	All samples were moistened to 40% of maximum water content with purified water and incubated in the dark at 20°C for up to 28 days.
Observations:	For measurements of short-term respiration, three aliquots of wet soil (40g) were sampled from one flask on each occasion, amended with a glucose/talc mixture and analysed for evolved CO_2 for between 17 and 22 hours. For measurements of nitrate and nitrite, three aliquots of wet soil (47g) were extracted with potassium chloride (2 M), centrifuged and filtered through filter paper. This extraction process was repeated and the combined aqueous extracts analysed for nitrate and nitrite by Flow Injection Analysis.
Data analysis:	Dixon's test, Dunnett's test.

Results:

Mean respiration and nitrogen concentrations data are presented in the tables below. Respiration rates measured in samples treated with CGA 205375 at 0.09 and 0.22 mg/kg did not deviate significantly from control values at any sampling time. Soil treated with dinoseb acetate showed reductions of up to 62% in respiration rates measured on days 0, 14 and 28.

Nitrite concentrations in control and CGA 205375-treated samples remained below the limits of detection for the duration of the study. Samples exposed to dinoseb acetate contained nitrite concentrations between 0.1 and 1.6 mg/kg soil. CGA 205375 treatment at 0.09 mg/kg did not cause more than 25% effect on nitrate concentrations measured at any sampling time. With the exception of values recorded on day 7, CGA 205375 treatment at 0.22 mg/kg did not have more than 25% effect on nitrate concentrations measured at any sampling time. The nitrate concentration of 0.22 mg/kg-treated soil on day 7 was 70% greater than in untreated soil, but declined to slightly below the control levels in the subsequent samples on days 14 and 28. Dinoseb acetate-treated soil, sampled on days 14 and 28, contained 53.2 and 74.2% more nitrate, respectively, than control samples, indicating a significant inhibitory effect on soil nitrification processes.

Table B.9.8.1-7: Effect of CGA 205375 and dinoseb acetate on short-term respiration rates in glucose-amended soil.

Treatment	Concentration (mg/ kg dry soil)	Incubation period (days)	Mean respiration rate (ml CO_2/h per kg dry soil)	Deviation from control (%)
Control	0	0	8.77	-
		7	6.34	-
		14	6.11	-
		28	6.26	-
CGA 205375	0.09	0	8.96	2.2
		7	6.60	4.1
		14	6.27	2.6
		28	6.39	2.1
	0.22	0	8.65	-1.5
		7	6.78	7.0
		14	5.90	-3.5
		28	6.30	0.6
Control	0	0	7.87	-
		7	5.81	-
		14	6.95	-
		28	5.44	-
Dinoseb acetate	25	0	5.10*	-35.2
		7	5.61*	-3.4
		14	3.29*	-52.7
		28	2.03*	-62.6

** significantly different from control ($p \leq 0.05$)*

Table B.9.8.1-8: Effect of CGA 205375 and dinoseb acetate on nitrite formation after amendment with lucerne meal.

Treatment	Concentration (mg/ kg dry soil)	Incubation period (days)	Nitrite		Nitrate	
			Mean NO_2^--N (mg NO_2^--N / kg dry soil)	Deviation from control (%)	Mean NO_3^--N (mg NO_3^--N / kg dry soil)	Deviation from control (%)
Control	0	0	<0.1	n.a.	8.4	-
		7	<0.1	n.a.	5.1	-
		14	<0.1	n.a.	6.2	-
		28	<0.1	n.a.	12.4	-
CGA 205375	0.09	0	<0.1	n.a.	7.8*	-7.1
		7	<0.1	n.a.	3.9*	-23.5
		14	<0.1	n.a.	6.0	-3.2
		28	<0.1	n.a.	12.9*	4.0
	0.22	0	<0.1	n.a.	7.8*	-7.1
		7	<0.1	n.a.	8.7*	70.6
		14	<0.1	n.a.	5.5*	-11.3
		28	<0.1	n.a.	11.8*	-4.8
Dinoseb acetate		0	0.1	n.a.	7.7*	-8.3
		7	0.2	n.a.	4.8	-5.9
		14	1.6	n.a.	9.5*	53.2
		28	0.1	n.a.	21.6*	74.2

**significantly different from control ($p \leq 0.05$)*

Exposure to CGA 205375 concentrations of 0.22 mg/kg for up to 28 days did not cause >25% effect on microbial respiration as measured by CO_2 evolution or nitrification processes as indicated by nitrite and nitrate concentrations.

RMS comments:

The study was well performed and reported, and is considered as valid for the risk assessment.

FORMULATED PRODUCTS

DIVIDEND 030FS

Notifier: No data was submitted on the effects of DIVIDEND 030FS to soil micro-organisms, since the notifier considered that this data requirement was covered by the tests on the active ingredient and on the other representative formulation.

RMS comments

The RMS agrees that the effects from this formulation can be considered to be covered by the data on the active ingredient. No further data is needed for Annex I inclusion.

SCORE 250EC

Reference:	**Maas, G (1990). Auswirkungen auf Aktivität der Bodenmikroflora nach Richtlinie Teil VI 1-1 (März 1987). Labor fur Bodenuntersuchungen UF, D3300 Braunschweig, Germany. Unpublished report no. 097. (Syngenta No. CGA 169374/0767). 02 October 1989 – 09 February 1990.**
Guideline:	BBA Teil VI 1-1 (März 1987).
GLP:	Yes.
Material and methods:	
Test substance:	CGD 96430F (equivalent to A-7402 A), containing 250 g difenoconazole/L; Batch number not stated.
Treatments:	Soil samples (two field soils) were mixed with A-7402 A to give nominal concentrations of 1.33 and 6.67 mg A-7402 A /kg soil. The test also incorporated untreated samples for use as control and samples treated with a toxic standard, dinoseb acetate. Samples intended for use in nitrification assessments were amended with ammonium sulfate prior to test substance application.
Duration:	28 days.
Test conditions:	After treatment, all samples were moistened to 60% of maximum water holding capacity and incubated at 20°C. Organic carbon contents of the soils were 0.95 and 2.42% respectively. Microbial biomass 151 and 913 mg/kg soil, pH 6.8 and 7.1.
Observations:	Three replicate samples were collected for measurements of dehydrogenase activity after 14 and 28 days. Nitrification was measured in three replicate samples collected on incubation days 7, 14, 21 and 28.
Data analysis:	Dixon's test, Dunnett's test.

Results:

Dehydrogenase activity and nitrogen concentration data are presented in the tables below. Dehydrogenase activity measured in samples treated with A-7402 A at 1.33 and 6.67 mg/kg did not deviate by more than 12% from control values at any sampling time. However, soil treated with dinoseb acetate showed reductions of up to 73% in activity measured on days 14 and 28.

Exposure to A-7402 A at 1.33 and 6.67 mg/kg did not have more than 25% effect on combined nitrate and nitrite concentrations or total nitrogen concentrations measured at any sampling time. Ammonium concentrations were affected by more than 25% on day 28 in Soil 1 treated at 1.33 mg/kg. However, this was not considered as treatment releated since it was not correlated with concentration or incubation time.

In soil 2, treatment with dinoseb-acetate treatment did not cause >25% effect on combined nitrate and nitrite concentrations whereas in Soil 1 treatment with dinoseb-acetate at 10 kg/ha caused up to 56% reductions in combined nitrate and nitrite concentrations in samples from days 7 and 14.

Table B.9.8.1-9: Effect of A-7402 A and dinoseb acetate on dehydrogenase activity

Treatment	Incubation period (days)	Dehydrogenase activity (mg TPF/ 100 g soil)	Percentage of control (%)	Dehydrogenase activity (mg TPF/ 100 g soil)	Percentage of control (%)
		Soil 1		Soil 2	
Control	14	4.203	n.a.	6.354	n.a.
	28	4.030	n.a.	5.934	n.a.
A-7402 A	14	3.756	89.4	7.122	112
1.33 mg/kg	28	4.162	103	5.558	93.7
A-7402 A	14	4.085	97.2	7.127	112
6.67 mg/kg	28	3.690	91.6	6.604	111
Dinoseb acetate	14	2.638	62.8	4.980	78.4
2 kg/ha	28	2.361	58.6	4.986	84.0
Dinoseb acetate	14	1.391	33.1	1.820	28.7
10 kg/ha	28	1.093	27.1	1.891	31.9

n.a. not applicable

Table B.9.8.1-10: Effect of A-7402 A and dinoseb acetate on nitrite formation in Soil 1

Treatment	Incubation period (days)	NH_4^+ (mg N/kg dry soil)	Percentage of control (%)	NO^2+ NO^3 (mg N/kg dry soil)	Percentage of control (%)	Total N (mg N/kg dry soil)	Percentage of control (%)
Control	7	2.471	n.a.	8.311	n.a.	10.782	n.a.
	14	0.129	n.a.	11.595	n.a.	11.724	n.a.
	21	0.126	n.a.	11.773	n.a.	11.899	n.a.
	28	0.363	n.a.	11.423	n.a.	11.786	n.a.
A-7402 A	7	2.676	108	8.318	100	10.993	102
1.33 mg/kg	14	0.119	92	11.496	99	11.615	99
	21	0.172	137	11.912	101	12.084	102
	28	0.238	66	11.819	103	12.075	102
A-7402 A	7	2.881	117	8.318	100	11.198	104
6.67 mg/kg	14	0.244	189	11.515	99	11.760	100
	21	0.198	157	11.951	102	12.150	102
	28	0.277	76	11.628	102	11.905	101
Dinoseb acetate	7	4.598	186	6.422	77	11.020	102
2 kg/ha	14	0.168	130	11.780	102	11.948	102
	21	0.132	105	11.872	101	12.004	101
	28	0.251	69	11.766	103	12.015	102
Dinoseb acetate	7	6.97	282	3.673	44	10.643	99
10 kg/ha	14	5.695	4415	5.801	50	11.496	98
	21	2.339	1856	9.415	80	11.753	99
	28	0.317	87	11.628	102	11.945	101

n.a. not applicable

Table B.9.8.1-11: Effect of A-7402 A and dinoseb acetate on nitrite formation in Soil 2

Treatment	Incubation period (days)	NH_4^+ (mg N/kg dry soil)	Percentage of control (%)	NO^2+ NO^3 (mg N/kg dry soil)	Percentage of control (%)	Total N (mg N/kg dry soil)	Percentage of control (%)
Control	7	0.126	n.a.	12.321	n.a.	12.448	n.a.
	14	0.112	n.a.	12.714	n.a.	12.826	n.a.
	21	0.042	n.a.	13.535	n.a.	13.577	n.a.
	28	0.456	n.a.	13.753	n.a.	14.209	n.a.
A-7402 A	7	0.105	83	12.518	102	12.623	101
1.33 mg/kg	14	0.042	38	13.093	103	13.135	102
	21	0.049	117	13.409	99	13.458	99
	28	0.533	117	13.584	99	14.118	99
A-7402 A	7	0.210	167	12.048	98	12.714	102
6.67 mg/kg	14	0.105	94	12.791	101	12.897	101
	21	0.070	167	13.233	98	13.304	98
	28	0.379	83	13.647	99	14.026	99
Dinoseb acetate	7	0.133	106	12.623	102	12.756	102
2 kg/ha	14	0.063	56	13.223	104	13.297	104
	21	0.035	83	13.718	101	13.753	101
	28	0.540	118	13.647	99	14.026	99
Dinoseb acetate	7	1.179	936	11.374	92	12.553	101
10 kg/ha	14	0.105	94	13.640	107	13.746	107
	21	0.147	350	14.132	104	14.279	105
	28	0.442	97	14.861	108	15.303	108

n.a. not applicable

Exposure to A-7402 A concentrations of 6.67 mg/kg (equivalent to 1.7 mg as/kg) for up to 28 days caused <25% effect on microbial respiration, as measured by dehydrogenase activity, and on nitrification processes.

RMS comments

The study was written in German. However, the study seemed to be well performed and reported and is considered as valid for the risk assessment.

B.9.8.2 Summary and risk assessment for soil micro-organisms

The available studies on effects of difenoconazole, metabolites in soil and the representative formulation SCORE 250EC are summarised in the table below. No study was submitted on the representative seed treatment formulation, DIVIDEND 030FS.

Table B.9.8.2-1: Effects of difenoconazole on nitrogen transformation and carbon mineralization

Type of study and time scale	Test soils	Dose range tested	Results	Reference
Nitrogen transformation				
ACTIVE INGREDIENT				
28 days	loamy sand silty loam	1.67 and 16.7 mg as/kg	Ca 60% increased NO_3 in Lucerne-amended loamy sand at both treatment levels at 28 d, less pronounced in NH_3SO_4-amended soil. <25% effect in silty loam	Ellgehausen (1990)
METABOLITE CGA 71019				
28 days	sandy loam	0.035 and 0.353 mg/kg	<25% effect after 28 days	Völkel (2000)
METABOLITE CGA 205375				
28 days	sandy loam	0.09 and 0.22 mg/kg	<25% effect after 28 days	Seyfried (2002)
DIVIDEND 030FS				
No data available.				
SCORE 250EC				
28 days	two field soils	0.33 and 1.67 mg as/kg	<25% effect after 28 days	Maas (1990)
Carbon mineralization				
ACTIVE INGREDIENT				
28 days	silty loam	1.67 and 16.7 mg as/kg	<25% effect in silty loam	Ellgehausen (1990)
METABOLITE CGA 71019				
28 days	sandy loam	0.035 and 0.353 mg/kg	<25% effect after 28 days	Völkel (2000)
METABOLITE CGA 205375				
28 days	sandy loam	0.09 and 0.22 mg/kg	<25% effect after 28 days	Seyfried (2002)
DIVIDEND 030FS				
No data available.				
SCORE 250EC				
28 days	two field soils	0.33 and 1.67 mg as/kg	<25% effect after 28 days	Maas (1990)
Single species test				
ACTIVE INGREDIENT				
Marasmius oraede (6d),	loamy sand	0.05 – 16.4 mg as/kg soil	6 d NOEC 1.64 mg as/kg	Grade (2000)
Mucor circinelloides (3d)	loamy sand	0.05 – 16.4 mg as/kg soil	3 d NOEC 4.9 mg as/kg	Grade (2000)
Paecilomyces marquandii (17d)	loamy sand	0.05 – 16.4 mg as/kg soil	17 d NOEC 16.4 mg as/kg	Grade (2000)
Phytophtora nicotianae (17d)	loamy sand	0.05 – 16.4 mg as/kg soil	17 d NOEC 16.4 mg as/kg	Grade (2000)

Risk assessments for soil micro-organisms at the representative uses of difenoconazole was provided by the notifier in **Document M-III, Section 6**. A summary is given below. The maximum predicted soil concentrations of difenoconazole and its metabolites, CGA 71019 and CGA 205375, following the representative use of difenoconazole were calculated as described in Document M-III, Section 5, and evaluated by the RMS in Annex B.8. The resulting maximum PEC values are listed in the table below.

Table B.9.8.2-2: Soil PEC values for difenoconazole, CGA 71019 and CGA 205375. Corrected based on RMS evaluation in Annex B.8.

Crop	Application rate (g as/ha)	Number of applications	Application interval (days)	Soil PEC (mg as/kg)		
				Difenoconazole	CGA 71019	CGA 205375
Pome fruit	75	4	7	0.136	0.005	0.012
Carrots	125	3	14	0.096	0.004	0.008
Seed treatment	60 mg as/kg seed	1	-	0.016	0.0006	0.0014

The notifier claimed that treatment with difenoconazole at 16.7 mg as/kg caused <25% effect on respiration or nitrification processes in soil while exposure to 4.9 mg/kg caused <25% effect on the growth of soil fungi. Therefore, difenoconazole was considered to have no biologically significant effect on soil micro-organisms at concentrations 35-fold greater than the maximum soil PEC of 0.136 mg/kg. Therefore, the notifier considered that difenoconazole would pose a low risk to soil micro-organisms.

Treatment with the soil metabolites CGA 71019 at 0.353 mg/kg caused <25% effect on soil respiration processes. Therefore, CGA 71019 had no biologically significant effect on soil micro-organisms at concentrations approximately 70-fold greater than the worst case maximum soil concentration of 0.005 mg/kg. Treatment with CGA 205375 at 0.22 mg/kg caused <25% effect on soil respiration processes. Therefore, CGA 205375 had no biologically significant effect on soil micro-organisms at concentrations approximately 20-fold greater than the maximum soil PEC of 0.012 mg/kg.

No data was submitted on the effects of the seed treatment formulation DIVIDEND 030FS to soil micro-organisms.

RMS comments and overall conclusion

The RMS noted that there seemed to be an >25% effect on nitrification (increase) after 28 days in one of the two soils tested with the active ingredient at 1.67 and 16.7 mg as/kg dw soil. However, the test concentrations were much higher than the expected PECsoil. At realistic exposure levels, it is considered likely that the effects would be less than 25% after 100 days, thus no further studies are needed for the representative use of SCORE 250EC.

For DIVIDEND 030FS, no data was submitted. However, the RMS agrees with the notifier that the effects from this formulation can be considered to be covered by the data on the active ingredient where a low risk was concluded.

Available studies are considered to fulfil the data requirements of Annex II and III of 91/414, and no further studies are needed for Annex I inclusion.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex IIA 8.6)

B.9.9.1 Insecticidal activity

Apart from the studies described on toxicity to non-target arthropods (see section B.9.5) and on effects on Collembola species (see section 9.7), no specific studies on insecticidal activity were submitted. The RMS considers that no further data is needed.

B.9.9.2 Herbicidal activity

ACTIVE INGREDIENT

Reference:	**Balluff, M. (2004). CGA 169374 (Difenoconazole): A toxicity test to determine the effects on seedling emergence and growth of three species of plants. GAB Biotechnologie GmbH, Niefern-Oschelbronn, Germany. Unpublished study number 20033067/S1-FGSE. (Syngenta File No 169374/2423)**
Guideline:	OECD: Proposal for Updating Guideline 208: Terrestrial (Non-Target) Plant Test: 208A: Seedling Emergence and Seedling Growth Test (July 2000).
GLP:	Yes.

Material and methods:

Test substance:	CGA 169374; Purity 94.3% w/w; Batch number WM 806228.
Species:	One monocot species; *Avena sativa,* and two dicot species; *Brassica napus* and *Glycine maxima.*
Treatments:	The test incorporated five test concentrations of CGA 169374 (0.1, 0.3, 1.0, 3.0 and 10 mg as/kg soil, equivalent to between 75 g and 7.5 kg as/ha), a solvent control treatment in which plants were treated with an acetonitrile/water mix and a negative control in which plants were treated with water. Spray solutions were prepared in acetonitrile/water applied to the soil (a sterilised soil/sand mix) with a hand atomiser at a rate equivalent to 300 L/ha. The soil was then mixed and transferred to plastic pots.
Number of plants:	The test incorporated 6 replicate pots containing 7 seeds, which were reduced to 5 seedlings after emergence for *A. sativa*, and 10 replicate pots of 5 seeds, which were reduced to 3 seedlings after emergence, for *B. napus* and *G. max*.
Duration:	21 days.
Test conditions:	Seeds of each species were sown in the pots and transferred to a glasshouse with a minimum day length of 16 hours, with additional lighting to maintain the intensity above 5000 lux. The average maximum/minimum air temperature in the glasshouse was 14.5/21.2°C. Plant pots were irrigated from below with water and nutrients, as necessary.
Observations:	Visual assessments of phytotoxicity were recorded after 7, 14 and 21 days. Seedling fresh weight per replicate was determined at test termination, 21 days after application. Analytical verification of the spray mixture concentrations were made at the beginning of the exposure phase.
Data analysis:	Dunnett's test, probit analysis.

Results:

Measured concentrations in the spray mixtures ranged from 64 to 108 % of nominal concentrations. The low value was determined for the spray solution that was used to prepare the 1 mg as/kg dose and was taken into account when considering the results.

Biomass data is presented in the table below. The results showed no adverse effect on the emergence of the species tested up to and including 10 mg as/kg. Similarly, exposure to CGA 169374 did not cause any visual

symptoms of toxicity. Exposure to CGA 169374 concentrations of 0.1 mg as/kg caused a significant 16% reduction in the biomass of *A. sativa* measured after 21 days. This effect increased to a 35% reduction in biomass at 10 mg as/kg. For *G. max,* concentrations up to and including 3 mg as/kg did not have a significant effect although exposure to 10 mg as/kg caused a 13% reduction in biomass. For *B. napus*, exposure to concentrations of 1 mg as/kg and higher caused 11-13% reductions in biomass.

Table B.9.9.2-1: Effect of exposure to CGA 169374 in soil on fresh weight at 21 days

Dose (mg as/kg)	*Avena sativa*		*Brassica napus*		*Glycine max*	
	Fresh weight (g)	**Inhibition (% reduction relative to control)**	**Fresh weight (g)**	**Inhibition (% reduction relative to control)**	**Fresh weight (g)**	**Inhibition (% reduction relative to control)**
0.0	3.75	-	4.15	-	5.26	-
Solvent	3.64	3.02	3.72	10.55	5.03	4.47
0.1	3.05 *	16.00	3.69	0.62	5.07	-0.78
0.3	2.53 *	30.31	3.67	1.32	4.98	0.90
1	2.44 *	33.01	3.23 *	12.94	4.92	2.17
3	2.38 *	34.43	3.26 *	12.33	4.79	4.67
10	2.36 *	35.08	3.30	11.14	4.38 *	12.89

** significant difference compared to the control (p > 0.05, Dunnett's test)*

The 21 day EC_{50} for CGA 169374 in *Avena sativa, Brassica napus* and *Glycine max* was considered to be >10 mg as/kg. NOEC was <0.1 mg as/kg dw for *Avena sativa*, 0.3 mg as/kg dw for *Brassica napus* and 3 mg as/kg dw for *Glycine maxima*.

RMS comments:

The study was well performed and reported and is considered to be valid for the risk assessment.

FORMULATED PRODUCTS

DIVIDEND 030FS

Due to its use as a seed-treatment, the notifier proposed that distribution of DIVIDEND 030FS within the crop environment is restricted and therefore, exposure of non-target flora and fauna other than those organism groups already assessed, can be considered to be negligible. The RMS agrees, and therefore further evaluation of effects on other non-target species is not considered necessary.

SCORE 250EC

Reference:	**Walder, L. (2000). Herbicide profiling test to evaluate the phytotoxicity of CGA 169374 250 EC (A-7402 G) to terrestrial non-target higher plants. Novartis Crop Protection, Stein, Switzerland. Unpublished report number SMQ 99003. (Syngenta No. CGA 169374/2029). 21 December 1999 – 11 January 2000.**
Guideline:	None.
GLP:	No.
Material and methods:	
Test substance:	A-7402 G containing 250 g CGA 169374/L. Batch number WM 901138.
Species:	*Beta vulgaris, Zea mays, Brassica napus, Avena fatua, Glycine max* and *Allium cepa*.

Treatments:	For the germination test, test substance application was made 24 hours after initial watering. For the vegetative vigour test, seedlings were grown for 14 days (cool climate species) or 17 days (warm climate species) prior to application. In both tests, stock solutions of A-7402 G prepared in deionised water were applied at 12.5, 25, 50, 100, 200 and 400 g formulation/ha using a track sprayer delivering 500 L/ha at 3 bar.
Number of plants:	3 seeds of *Z. mays* and *G. max,* 5 seeds of *B. vulgaris,* 6 seeds of *A. fatua* and 20 seeds of *B. napus* and *A. cepa.* Both tests incorporated two replicate trays for each treatment rate and an untreated control.
Duration:	14 days after test substance application for the seedling emergence test and 21 days after treatment for the vegetative vigour test.
Test conditions:	Seeds were sown in a clay loam soil in plastic trays, watered and transferred to glasshouse conditions with a minimum daylength of 14 hours and additional lighting whenever daylight intensity dropped below 10 000 lux. Cool season species (*B. napus, B. vulgaris, A. fatua* and *A. cepa*) were maintained with minimum day/night temperatures of 15/20°C while warm season species (*Z. mays* and *G. max*) were maintained at 18/25°C.
Observations:	Visual assessments of phytotoxicity were recorded 14 days after test substance application for the seedling emergence test and 21 days after treatment for the vegetative vigour test. In both cases phytotoxicity was rated on a scale of 1-9 where a score of 1 indicates complete necrosis or inhibition of germination while 9 indicates vigourous growth comparable to the growth of control plants.
Data analysis:	Not stated, not needed.

Results:

Seedling emergence and vegetative vigour data are presented in the tables below.

In the vegetative vigour test, A-7402 G concentrations up to 400 g formulation/ha caused <50% effect on the growth of those species tested. In the seedling emergence test, five of the six species tested showed less than 50% reduction in emergence/vigour at concentrations up to 400 g formulation/ha. In the case of *Glycine max* effects of more than 50% decrease were seen at 400 g/ha but <25% effect was seen at 200 g/ha.

Table B.9.9.2-2: Effect of A-7402 G on seedling emergence assessed 14 days after treatment on a scale of 1 – 9, where a score of 1 indicates complete necrosis or inhibition of germination while 9 indicates vigourous growth comparable to the growth of control plants.

Species	Application rate (g formulation/ha)					
	400	200	100	50	25	12.5
Brassica napus	9	9	9	9	9	9
Avena fatua	9	9	9	9	9	9
Beta vulgaris	9	9	9	9	9	9
Zea mays	8	9	9	9	9	9
Glycine max	5	7.5	8	8	8	9
Allium cepa	9	9	9	9	9	9

Table B.9.9.2-3: Effect of A-7402 G on vegetative vigour assessed 21 days after treatmenton a scale of 1 – 9, where a score of 1 indicates complete necrosis or inhibition of germination while 9 indicates vigourous growth comparable to the growth of control plants.

Species	Application rate (g formulation/ha)					
	400	200	100	50	25	12.5
Brassica napus	9	9	9	9	9	9
Avena fatua	9	9	9	9	9	9
Beta vulgaris	9	9	9	9	9	9
Zea mays	7.5	8	8	8	8	9
Glycine max	7	8	8.5	8.5	8.5	9
Allium cepa	9	9	9	9	9	9

With the exception of seedling emergence of *Glycine max*, the EC_{50} for all species tested was >400 g formulation/ha (100 g as/ha). For *Glycine max*, the EC_{50} is considered to be approximately 400 g formulation/ha (100 g as/ha). NOEC could be set to 12.5 g formulation/ha (3.1 g as/ha).

RMS comments

Although the study was not a GLP-study, it was well performed and reported, and is considered as valid for the risk assessment.

B.9.9.3 Summary and risk assessment for non-target flora and fauna believed to be at risk

A summary of available data on the effects of difenoconazole on non-target plants is given in the table below.

Table B.9.9.3-1: Summary of results from non-target plant studies with difenoconazole and SCORE 250EC.

Species	Test type	Rate (g as/ha)	Result	Reference
LABORATORY STUDIES				
ACTIVE INGREDIENT				
Avena sativa	soil incorporation	0.1, 0.3, 1.0, 3.0 and 10 mg as/kg soil, equivalent to between 75 g and 7.5 kg as/ha	21 day ER_{50} >10 mg as/kg dw soil based on fresh biomass	**Balluff (2004)**
Brassica napus	soil incorporation	0.1, 0.3, 1.0, 3.0 and 10 mg as/kg soil, equivalent to between 75 g and 7.5 kg as/ha	21 day ER_{50} >10 mg as/kg dw soil based on fresh biomass	**Balluff (2004)**
Glycine max.	soil incorporation	0.1, 0.3, 1.0, 3.0 and 10 mg as/kg soil, equivalent to between 75 g and 7.5 kg as/ha	21 day ER_{50} >10 mg as/kg dw soil based on fresh biomass	**Balluff (2004)**
DIVIDEND 030FS				
no data available, not needed				
SCORE 250EC				
Brassica napus	spray application	12.5, 25, 50, 100, 200 and 400 g formulation/ha	21 day ER_{50} >100 g as/ha based on seedling emergence 14 day ER_{50} >100 g as/ha based on vegetative vigour	**Walder (2000)**
Avena fatua	spray application	12.5, 25, 50, 100, 200 and 400 g formulation/ha	21 day ER_{50} >100 g as/ha based on seedling emergence 14 day ER_{50} >100 g as/ha based on vegetative vigour	**Walder (2000)**

Species	Test type	Rate (g as/ha)	Result	Reference
Beta vulgaris	spray application	12.5, 25, 50, 100, 200 and 400 g formulation/ha	21 day ER_{50} >100 g as/ha based on seedling emergence 14 day ER_{50} >100 g as/ha based on vegetative vigour	**Walder (2000)**
Zea mays	spray application	12.5, 25, 50, 100, 200 and 400 g formulation/ha	21 day ER_{50} >100 g as/ha based on seedling emergence 14 day ER_{50} >100 g as/ha based on vegetative vigour	**Walder (2000)**
Glycine max	spray application	12.5, 25, 50, 100, 200 and 400 g formulation/ha	21 day ER_{50} 100 g as/ha based on seedling emergence 14 day ER_{50} >100 g as/ha based on vegetative vigour	**Walder (2000)**
Allium cepa	spray application	12.5, 25, 50, 100, 200 and 400 g formulation/ha	21 day ER_{50} >100 g as/ha based on seedling emergence 14 day ER_{50} >100 g as/ha based on vegetative vigour	**Walder (2000)**

A risk assessment was provided by the notifier in Document MIII, Section 6). Asummary is given below.

Non-target plants and seeds present in off-crop areas may be exposed to SCORE 250EC by spray drift onto their foliage. Alternatively, non-target plant seed, that has been shed, may be exposed by spray drift onto the soil. For risk assessment purposes, the maximum exposure concentrations were estimated using BBA drift values at varying distances from the crop edge. For effects on seed, the total drift rate was assumed to be deposited on soil and for effects on emerged vegetation, the total drift rate was assumed to be intercepted by the vegetation as a worst case.

Table B.9.9.3-2: Soil and foliar predicted environmental residues of difenoconazole for NTP risk assessment

Crop	Pome fruit	Carrots
Distance from crop	**3 m**	**1 m**
Maximum single application rate (g as/ha)	75	125
Drift rate (%)	15.7	2.77
PER (g as/ha)	12	3.5

In glasshouse tests with A-7402 G, applications of 400 g formulation/ha (95.7 g as/ha assuming a formulation density of 1.045 kg/L) caused <25% effect on seedling emergence or vegetative vigour in five of the six species tested. In the case of soyabean (*Glycine max*), treatment with 400 g formulation/ha caused <50% in the vegetative vigour test but had approximately 50% effect in the seedling emergence test. Effects from this study were expressed in terms of reduction in visual score based on observations of plant health and size following exposure to pre-emergence applications of A-7402 G. However, in a further germination study where technical difenoconazole was incorporated into the soil before the seed was sown (**Balluff, 2004**), rates up to 10 mg as/kg (equivalent to 7.5 kg as/ha) caused a maximum 35% (i.e. <50%) effect in soya bean. This effect was estimated from assessments of fresh weight, which according to the notifier are considered a better indicator of effects on plant growth than visual scores. Nevertheless, for the purposes of risk assessment, the minimum EC_{50} for vegetative vigour and seedling emergence was considered to be 100 g as/ha.

According to **SANCO/10329/2002,** risk to terrestrial plants is considered acceptable provided that the maximum single application rate causes <50% effect on plant growth. As the maximum rate of 125 g as/ha was not tested, the potential risk of SCORE 250EC to non-target plants was evaluated from toxicity exposure ratios based on exposure via drift (PER) and EC_{50} values calculated as follows:

$$TER = \frac{EC_{50}\ (\text{g ai/ha})}{PER\ (\text{g ai/ha})}$$

TER values were calculated using the minimum EC_{50} of 95.7 g as/ha estimated from seedling emergence and vegetative vigour tests conducted with 6 test species. Resulting TERs are presented in the table below and were compared to a TER trigger of 5 for datasets based on 6 species or more.

Table B.9.9.3-3: TER values for non-target terrestrial plants

Test type	Species	EC_{50} (g as/ha)	Pome fruit		Carrots	
			3 m from crop		1 m from crop	
			PER (g as/ha)	TER	PER (g as/ha)	TER
Seedling emergence	*Glycine max*	95.7	12	8.1	3.5	28
Vegetative vigour	All species tested	95.7	12	8.1	3.5	28

Vegetative vigour and seedling emergence TER values exceed the recommended trigger of 5, indicating that SCORE 250EC poses low risk to the vegetative growth and seedling emergence of off-crop non-target terrestrial plants.

RMS comments

The RMS agrees with the risk assessment provided by the notifier. In conclusion, the risk to off-crop non-target terrestrial plants is assessed to be low at the representative uses of difenoconazole for seed treatment in cereals and at spray application in pome fruit and carrots. No further data is needed.

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

B.9.10.1 Effect study

Reference:	**Grade, R. (1997) Report on the test for activated sludge respiration inhibition of CGA 169374 tech. Novartis Crop Protection AG, Switzerland. Unpublished report no. 973535. (Syngenta File No 169374/1422)**
Guideline:	OECD 209 (1984); EEC L133 s. 118-122 (1988).
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number P.807002, purity 91%.
Treatments:	Activated sludge was exposed to difenoconazole at nominal concentrations of 1, 3.2, 10, 32 and 100 mg/L. The test incorporated a dose response to a reference standard (3,5-dichlorophenol) at five nominal concentrations of 1, 3.2, 10, 32 and 100 mg/L, and an untreated control.

Number of replicates:	2 replicates per treatment level, toxic standard and control.
Duration:	3 hours.
Test conditions:	The activated sludge was obtained from a sewage treatment plant at CH-4153 Reinach, Switzerland, 1 day prior to study initiation. After settling, the sludge (80 mL) was transferred to 250 mL flasks and mixed with 120 mL of dilute nutrient solution containing the test substance. The sludge concentration in each flask was 1.75 g suspended solids/L. The samples were kept at 21±1°C and continuous aeration.
Observations:	The respiration rate of each culture was determined by measuring oxygen uptake with an electrode for 10 minutes. The respiration rates of treated cultures were expressed as a percentage of the mean rates seen in control cultures.
Data analysis:	Linear regression.

Results:

The effects of difenoconazole and the reference substance, 3,5-dichlorophenol, on respiration rates of activated sewage sludge are presented in the table below. While difenoconazole concentrations of 1 and 3.2 mg/L did not affect respiration rates, concentrations of 10, 33 and 100 mg/L caused 4, 9 and 18% inhibition, respectively. Inhibition values below 10% are within expected experimental variability and are not considered to be a consequence of exposure to the test substance. 3,5-DCP concentrations of 3.2, 10 and 32 mg/L caused 9, 50 and 78% reductions in the rate of oxygen consumption thus confirming that the activated sludge was responding normally and contained viable sludge organisms.

Table B.9.10.1-1: Effect of difenoconazole on oxygen consumption of activated sludge.

Test substance	Concentration (mg/L)	O_2 consumption rate (mg/L/h)	Percent inhibition
None	Control	46.2	1
	Control	46.9	-1
Difenoconazole	1.0	47.1	-1
	3.2	47.1	-1
	10	44.4	4
	32	42.1	9
	100	38.1	18
3,5-DCP	3.2	42.4	9
	10.0	23.1	50
	32.0	10.2	78

Based on nominal concentrations, the NOEC and EC_{50} for difenoconazole were estimated to be 32 and >100 mg/L, respectively.

RMS comments:

The study was well performed and reported, and is considered as valid for the risk assessment.

B.9.10.2 Risk assessment for biological methods of sewage treatment

For the proposed uses of difenoconazole contamination of sewage treatment plants is not considered likely. Further, with a NOEC of 32 mg as/L and an EC_{50} of >100 mg/L, the risk for harmful effects on biological methods of sewage treatment is considered to be acceptable.

B.9.11 References relied on

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
Annex II data and information					
KIIA 8.1.1/01	Fletcher, D.W.	1988a	21-day acute oral LD50 study with CGA 169374 technical in Mallard ducks Novartis Crop Protection AG, Basel, Switzerland [redacted] GLP Not Published Syngenta File N° CGA169374/0014	N	SYN
KIIA 8.1.1/02	Leopold, M.A.	1993	Acute oral toxicity study with CGA 169374 technical in Japanese Quail Novartis Crop Protection AG, Basel, Switzerland [redacted] GLP Not Published Syngenta File N° CGA169374/0843	N	SYN
KIIA 8.1.2/01	Fletcher, D.W.	1988b	11-day acute dietary LC50 study with CGA 169374 in Mallards ducklings Novartis Crop Protection AG, Basel, Switzerland [redacted] GLP Not Published Syngenta File N° CGA169374/0290	N	SYN
KIIA 8.1.2/02	Fletcher, D.W.	1988c	9-Day acute dietary LC50 study with CGA 169374 techn. in Bobwhite quail Novartis Crop Protection AG, Basel, Switzerland [redacted] GLP Not Published Syngenta File N° CGA169374/0332	N	SYN
KIIA 8.1.2/03	Beavers, B., Jaber, M.	1983a	A dietary LC50 in the Mallard with CGA 131013. Novartis Crop Protection AG, Basel, Switzerland [redacted] Report No 108-222 Not GLP Not Published Syngenta File N° CGA131013/0034	N	TDMG

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
KIIA 8.1.2/04	Beavers, B., Jaber, M.	1983b	A dietary LC50 in the Bobwhite with CGA 131013. Novartis Crop Protection AG, Basel, Switzerland [redacted] Report No 108-221 Not GLP Not Published Syngenta File N° CGA131013/0033	N	TDMG
KIIA 8.1.3/01	Pedersen, C.A.	1990	CGA 169374 techn.: Toxicity and reproduction study in Mallard ducks Novartis Crop Protection AG, Basel, Switzerland [redacted] Report No 88-DR-3 GLP Not Published Syngenta File N° CGA169374/0015	N	SYN
KIIA 8.1.3/02	Frey, L.T.	2000	A reproduction study with the Northern Bobwhite Novartis Crop Protection AG, Basel, Switzerland [redacted] Report No 108-427 GLP Not Published Syngenta File N° CGA169374/2065	Y	SYN
KIIA 10.1/01	Bartlett, D	2006	Radiolabel study to investigate systemicity of Difenoconazole from Dividend seed treatment. Jealotts Hill International, UK report no. FP 04 1001 Unpublished. Not GLP Syngenta File No CGA169374/2803	N	SYN
KIIA 10.1/02	Kilgour, J	2004	Toxicological relevance of plant metabolite CGA131013. Syngenta Central Toxicology Laboratory, UK. Unpublished. Not GLP. Syngenta File No. CGA131013/0046	N	SYN
KIIA 8.2.1/02	Surprenant, D.C.	1990a	Acute toxicity of CGA 169374 to Rainbow trout under flow-through conditions Novartis Crop Protection AG, Basel, Switzerland [redacted] Report No 88-5-2663 GLP Not Published Syngenta File N° CGA169374/0333	N	SYN

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KIIA 8.2.1/03	Bowman, J.H.	1988	Acute toxicity of CGA 169374 technical to Bluegill sunfish (lepomis macrochirus) Novartis Crop Protection AG, Basel, Switzerland [redacted] Report No 34834 GLP Not Published Syngenta File N° CGA169374/0016	N	SYN
KIIA 8.2.1/05	Machado, M.W.	1993	Acute toxicity to Sheepshead minnow (cyprinidon variegatus) under Flow-Through conditions Novartis Crop Protection AG, Basel, Switzerland [redacted] Report No 93-5-4795 GLP Not Published Syngenta File N° CGA169374/0949	N	SYN
KIIA 8.2.1/06	Rufli, H.	1983	Report on the test for acute toxicity of CGA 98032 to rainbow trout. Novartis Crop Protection AG, Basel, Switzerland [redacted] No 821418 Not GLP Not Published Syngenta File N° CGA71019/0024	N	TDMG
KIIA 8.2.1/07	Swarbrick, R.H.	2001a	Acute toxicity to rainbow trout (Oncorhynchus mykiss) Syngenta Crop Protection AG, Basel, Switzerland [redacted] United Kingdom, Report No BL7201/B GLP Not Published Syngenta File N° CGA205375/0014	Y	SYN
KIIA 8.2.2/01	Grade, R.	1993a	Report on the prolonged toxicity test of CGA 169374 tech. to rainbow trout Novartis Crop Protection AG, Basel, Switzerland [redacted] Report No 938152 GLP Not Published Syngenta File N° CGA169374/0859	N	SYN

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KIIA 8.2.2/02	Dorgerloh, M. and Sommer H.	2002	1,2,4-Triazole: Juvenile Growth Test on Fish (Oncorhychus mykiss) Syngenta Crop Protection AG, Basel, Switzerland [redacted] GLP Not Published Syngenta File N° CGA71019/0052	Y	TDMG
KIIA 8.2.2.1/01	Surprenant, D.C.	1987b	Toxicity of CGA 169374 to Fathead minnow (pimephales promelas) embryos and larvae Novartis Crop Protection AG, Basel, Switzerland [redacted] GLP Not Published Syngenta File N° CGA169374/0018	N	SYN
KIIA 8.2.2.1/02	Surprenant, D.C.	1990b	CGA 169374 techn. Toxicity to fathead minnow (Pimephales promelas) embryos and larvae Novartis Crop Protection AG, Basel, Switzerland [redacted] GLP Not Published Syngenta File N° CGA169374/0020	N	SYN
KIIA 8.2.3/01	Forbis, A.D.	1987	Uptake, depuration and bioconcentration of 14C-CGA 169374 by Bluegill sunfish (lepomis macrochirus) Novartis Crop Protection AG, Basel, Switzerland [redacted], Report No 34837 GLP Not Published Syngenta File N° CGA169374/0036	N	SYN
KIIA 8.2.3/02	Fackler, P.H.	1992	Bioconcentration and Elimination of 14C-Residues by Bluegill (Lepomis macrochirus) Exposed to CGA 169374 Novartis Crop Protection AG, Basel, Switzerland [redacted] Not Published Syngenta File N° CGA169374/0531	N	SYN

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KIIA 8.2.4/01	Forbis, A.D.	1988a	Acute toxicity of CGA 169374 to Daphnia magna Novartis Crop Protection AG, Basel, Switzerland ABC Analytical Bio-Chemistry Lab. Inc., Columbia, United States, Report No 34835 GLP Not Published Syngenta File N° CGA169374/0021	N	SYN
KIIA 8.2.4/02	Surprenant, D.C.	1990c	CGA 169374 techn., Acute toxicity to mysid shrimp (Mysidopsis bahia) under flow-through conditions Novartis Crop Protection AG, Basel, Switzerland Springborn Laboratories Inc., Wareham, United States, Report No SLI-89-2-2936 GLP Not Published Syngenta File N° CGA169374/0023	N	SYN
KIIA 8.2.4/03	Surprenant, D.C.	1990d	CGA 169374 techn., Acute toxicity to Eastern oysters (Crassostrea virginica) under flow-through conditions Novartis Crop Protection AG, Basel, Switzerland Springborn Laboratories Inc., Wareham, United States, Report No SLI-89-05-2988 GLP Not Published Syngenta File N° CGA169374/0024	N	SYN
KIIA 8.2.4/04	Bell, G	1995	Fluquinconazole Technical material 100.8% w/w 1,2,4 Triazole: Acute Toxicity to Daphnia magna Syngenta Crop Protection AG, Basel, Switzerland Huntingdon Life Sciences Ltd., Huntingdon, United Kingdom, Report No ENVIR/95/52 GLP Not Published Syngenta File N° CGA169374/2320	N	SYN
KIIA 8.2.4/05	Swarbrick, R H	2002	Acute toxicity to Daphnia magna Syngenta Crop Protection AG, Basel, Switzerland Brixham Environmental Laboratory, Brixham, United Kingdom, Report No BL7202/B GLP Not Published Syngenta File N° CGA205375/0012	Y	SYN

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KIIA 8.2.5/01	Forbis, A.D.	1988b	Chronic toxicity of CGA 169374 to Daphnia magna under flow-through test conditions Novartis Crop Protection AG, Basel, Switzerland ABC Analytical Bio-Chemistry Lab. Inc., Columbia, United States, Report No 34836 GLP Not Published Syngenta File N° CGA169374/0022	N	SYN
KIIA 8.2.6/01	Rufli, H.	1989	Alga Growth inhibition test of CGA 169374 to Green Algae(Scenedesmus supsicatus) Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Ltd., Basel, Switzerland, Report No 881699 GLP Not Published Syngenta File N° CGA169374/0026	N	SYN
KIIA 8.2.6/02	Grade, R.	1993b	Report on the growth inhibition test of CGA 169374 tech. to green algae (Scenedesmus subspicatus) Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Basel, Oekotoxikologie, Basel, Switzerland, Report No 938153 GLP Not Published Syngenta File N° CGA169374/0860	N	SYN
KIIA 8.2.6/03	Palmer, S.J., Kendall, T.Z., Krueger, H.O.	2001	1,2,4-triazole: a 96-hour toxicity test with the freshwater alga (Selenastrum capricornutum) Syngenta Crop Protection AG, Basel, Switzerland Wildlife International Ltd., Easton, MD, United States, Report No 528A-101 GLP Not Published Syngenta File N° CGA71019/0044	Y	TDMG
KIIA 8.2.6/04	Swarbrick, R.H.	2001b	CGA205375: Toxicity to the green alga Selenastrum capricornutum Syngenta Crop Protection AG, Basel, Switzerland Brixham Environmental Laboratory, Brixham, United Kingdom, Report No BL7203/B GLP Not Published Syngenta File N° CGA205375/0015	Y	SYN

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KIIA 8.2.7/01	van, der Kolk J.	1999	Chronic effects on Midge Larvae (Chironomus riparius) in a water / sediment system Novartis Crop Protection AG, Basel, Switzerland Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland, Report No 97-192-1008 GLP Not Published Syngenta File N° CGA169374/1816	Y	SYN
KIIA 8.2.7/02	Grade, R.	2001	Toxicity test of CGA 211391 (metabolite of CGA 169374) on sediment-dwelling Chironomus riparius (syn. Chironomus thummi) under static conditions Syngenta Crop Protection AG, Basel, Switzerland, Report No 2003511 GLP Not Published Syngenta File N° CGA211391/0001	Y	SYN
KIIA 8.3.1.1/01	Hoxter, K.A., Jaber, M.	1989	Acute contact toxicity study with the honey bee Novartis Crop Protection AG, Basel, Switzerland Wildlife International Ltd., Easton, MD, United States, Report No 108-302A GLP Not Published Syngenta File N° CGA169374/0028	N	SYN
KIIA 8.3.1.1/02	Greig-Smith, P.W.	1990	Acute contact and oral toxicity of CGA 169374 to honeybees Novartis Crop Protection AG, Basel, Switzerland ADAS, Central Science Lab., Tolworth, United Kingdom, Report No C89-0370 GLP Not Published Syngenta File N° CGA169374/0029	N	SYN
KIIA 8.4.1/01	Surprenant, D.C.	1987c	Fourteen-day toxicity test exposing earthworm (eisenia foetida) to CGA 169374 Novartis Crop Protection AG, Basel, Switzerland Springborn Laboratories Inc., Wareham, United States, Report No 87-9-2494 GLP Not Published Syngenta File N° CGA169374/0027	N	SYN

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KIIA 8.4.1/02	Heimbach, F.	1986	Acute toxicity of 1,2,4-triazole (technical) to earthworms. Novartis Crop Protection AG, Basel, Switzerland Bayer AG, Leverkusen, Germany, Report No HBF/RG 59 GLP Not Published Syngenta File N° CGA71019/0021	N	SYN
KIIA 8.4.1/03	Bätscher, R	2002	Acute Toxicity of CGA 205375 (Metabolite of CGA 169374) to the Earthworm Eisenia fetida in a 14-day Test Syngenta Crop Protection AG, Basel, Switzerland RCC Ltd., Itingen, Switzerland, Report No 812092 GLP Not Published Syngenta File N° CGA205375/0011	Y	SYN
KIIA 8.4.2/03	Ehlers, H.A.	2000	Effects of 1,2,4-triazole on reproduction and growth of Earthworms Eisenia fetida (Savigny 1826) in artificial soil Novartis Crop Protection AG, Basel, Switzerland IBACON GmbH, Rossdorf, Germany, Report No 7781022 GLP Not Published Syngenta File N° CGA64250/4385	Y	TDMG
KIIA 8.4.2/01	Coulson, M	2006	Re-analysis of CGA169374 Sub-lethal Earthworm Study [CGA169374/1926]. Jealotts Hill International Report. Unpublished Not GLP Syngenta File No. CGA169374/2812	N	SYN
KIIA 8.5/01	Ellgehausen, H.	1990	The effects of CGA 169374 on the activity of soil microbes Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Ltd., Basel, Switzerland, Report No 17-90 GLP Not Published Syngenta File N° CGA169374/0289	N	SYN
KIIA 8.5/02	Grade, R.	2000	The effect of CGA 169374 tech. on the growth of soil fungi on soil-maltextract-agar-plates Novartis Crop Protection AG, Basel, Switzerland, Report No 203636 GLP Not Published Syngenta File N° CGA169374/2042	Y	SYN

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KIIA 8.5/03	Völkel, W.	2000	The effects of CGA 71019 on soil respiration and nitrification Novartis Crop Protection AG, Basel, Switzerland RCC Ltd., Itingen, Switzerland, Report No 763367 GLP Not Published Syngenta File N° CGA71019/0042	Y	TDMG
KIIA 8.5/04	Seyfried, B	2002	The Effects of CGA 205375 (Metabolite of CGA 169374) on Soil Respiration and Nutrification Syngenta Crop Protection AG, Basel, Switzerland RCC Ltd., Itingen, Switzerland, Report No 808176 GLP Not Published Syngenta File N° CGA205375/0019	Y	SYN
KIIA 8.6/01	Meister, A	2002	Effects of CGA 169374 on Reproduction of the Collembola Folsomia candida in Artificial Soil Syngenta Crop Protection AG, Basel, Switzerland IBACON GmbH, Rossdorf, Germany, Report No 8491016 GLP Not Published Syngenta File N° CGA169374/2202	Y	SYN
KIIA 8.6/02	Moser, Th. and Scheffczyk A.	2002	1,2,4-Triazole: Acute and Reproduction Toxicity to the Collembolan species Folsomia candida Syngenta Crop Protection AG, Basel, Switzerland ECT Oekotoxikologie GmbH, Bad Soden am Ts., Germany, Report No P31CR GLP Not Published Syngenta File N° CGA71019/0053	Y	TDMG
KIIA 8.6/03	Balluff, M.	2004	CGA169374 (Difenconazole): A toxicity test to determine the effects on seedling emergence and growth of three species of plants Syngenta Crop Protection AG, Basel, Switzerland GAB Biotechnologie GmbH, Niefern, Germany, Report No 20033067/S1-FGSE GLP Not Published Syngenta File N° CGA169374/2423	Y	SYN

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
KIIA 8.7/01	Grade, R.	1997	Report on the test for activated sludge respiration inhibition of CGA 169374 Novartis Crop Protection AG, Basel, Switzerland Novartis Crop Protection AG, Basel, Switzerland, Report No 973535 GLP Not Published Syngenta File N° CGA169374/1422	N	SYN
Annex III data and information – DIVIDEND 030FS					
KIIIA2 10.1.3/01	Gallagher, S.P., Beavers, J.B.	1999	A-9142 G A test for avoidance of treated wheat seed with the rock dove (Columba livia) Novartis Crop Protection AG, Basel, Switzerland Wildlife International Ltd., Easton, MD, United States, Report No 108-416 GLP Not Published Syngenta File N° CGA169374/1947	N	SYN
KIIIA 10.1/01	Bartlett, D	2006	Radiolabel study to investigate systemicity of Difenoconazole from Dividend seed treatment. Jealotts Hill International, UK report no. FP 04 1001 Unpublished. Not GLP Syngenta File No CGA169374/2803	N	SYN
KIIIA 10.1/02	Murfitt, R	2006	Investigation of Crop Seedling Shoot Weights at Differing Timings after Emergence Jealotts Hill International, UK Report. Unpublished Not GLP Syngenta File No N/1048	N	SYN
KIIIA2 10.2.1/02	Gries, Th.	1999b	Acute Toxicity test of CGA 169374 FS 30 (A9142 G) to daphnids (Daphnia magna) under static conditions Novartis Crop Protection AG, Basel, Switzerland Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland, Report No 1047.062.110 GLP Not Published Syngenta File N° CGA169374/1908	N	SYN

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KIIIA2 10.2.1/03	Gries, Th.	1999c	Toxicity test of CGA 169374 FS 30 (A9142 G) to the freshwater algae Pseudokirchneriella subcapitata Novartis Crop Protection AG, Basel, Switzerland Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland, Report No 1047.062.430 GLP Not Published Syngenta File N° CGA169374/1906	N	SYN
KIIIA2 10.5.1/01	Kleiner, R.	2000	Acute dose-response toxicity of CGA 169374 EC250 (A-7402 G) to the cereal aphid parasitoid Aphidius rhopalosiphi (Destefani-Perez) under laboratory conditions Novartis Crop Protection AG, Basel, Switzerland BioChem GmbH, Cunnersdorf, Germany, Report No 99 10 48 083 GLP Not Published Syngenta File N° CGA169374/2095	Y	SYN
KIIIA2 10.5.1/02	Kleiner, R.	2001	Acute dose-response toxicity of CGA 169374 EC 250 (A-7402 G) to the predatory mite Typhlodromus pyri (SCHEUTEN) under laboratory conditions Syngenta Crop Protection AG, Basel, Switzerland BioChem GmbH, Cunnersdorf, Germany, Report No 99 10 48 084 GLP Not Published Syngenta File N° CGA169374/2131	Y	SYN
KIIIA2 10.5.1/03	Grimm, C.	1999a	Acute toxicity test of CGA 169374 FS 30 (A-9142 G) to the rove beetle Aleochara bilineata Gyll. (Coleoptera, Staphylinidae) Novartis Crop Protection AG, Basel, Switzerland, Report No 982924 GLP Not Published Syngenta File N° CGA169374/1959	N	SYN
KIIIA2 10.5.1/04	Grimm, C.	1999b	Acute toxicity test of CGA 169374 FS 30 (A-9142 G) to the predatory ground beetle Poecilus cupreus L. (Coleoptera: Carabidae) Novartis Crop Protection AG, Basel, Switzerland, Report No 982923 GLP Not Published Syngenta File N° CGA169374/1960	N	SYN

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KIIIA2 10.5.1/05	Reber, B.	1999	Acute toxicity of CGA 169374 FS 30 (A-9142 G) to larvae of the predatory ground beetle Poecilus cupreus L. (Coleoptera: carabidae) Novartis Crop Protection AG, Basel, Switzerland, Report No 991501 GLP Not Published Syngenta File N° CGA169374/1975	N	SYN
KIIIA2 10.6.1.2/01	Friedrich, S	2002	Sublethal toxicity of CGA169374 FS 030 (A-9142 G) to the earthworm Eisenia fetida Syngenta Crop Protection AG, Basel, Switzerland BioChem agrar, Gerichshain, Germany, Report No 021048036 GLP Not Published Syngenta File N° CGA169374/2242	Y	SYN
KIIIA2 10.6.2/01	Meister, A. and Schwiening S.	2002	Effects of A7402G on the Decomposition of Organic Material enclosed in Litter Bags in the field. Syngenta Crop Protection AG, Basel, Switzerland IBACON GmbH, Rossdorf, Germany, Report No 8381081 GLP Not Published Syngenta File N° CGA169374/2228	Y	SYN
Annex III data and information – SCORE 250EC					
KIIIA 10.1/03	Kilgour, J	2004	Toxicological relevance of plant metabolite CGA131013. Syngenta Central Toxicology Laboratory, UK. Unpublished. Not GLP. Syngenta File No. CGA131013/0046	N	SYN
KIIIA1 10.2.1/03	Voigt, H.	1991	Toxizität von CGD 96 430 F für Regenbogenforellen Salmo gairdnerii (21 Tage) Novartis Crop Protection AG, Basel, Switzerland [redacted] No 01/91/299 Not GLP Not Published Syngenta File N° CGA169374/0762	N	SYN

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KIIIA1 10.2.1/04	Voigt, H.	1990b	Toxizität von CGD 96 430 F auf Wasserflöhe (Daphnia magna) (48 H) Novartis Crop Protection AG, Basel, Switzerland Oekolimna, Burgwedel, Germany, Report No 07/90/217 GLP Not Published Syngenta File N° CGA169374/0759	N	SYN
KIIIA1 10.2.1/07	Peters, A.	1992	CGA 169374 Toxizität von CGD 96430 F auf Scenedesmus subspicatus CHODAT (96 h) Novartis Crop Protection AG, Basel, Switzerland Oekolimna, Burgwedel, Germany, Report No N/A GLP Not Published Syngenta File N° CGA169374/0630	N	SYN
KIIIA1 10.2.1/09	Neumann, Ch.	1997	Acute toxicity test of CGA 169374 EC 250 (A-7402 G) on sediment-dwelling larvae of Chironomus riparius (syn. Chironomus thummi) under static conditions Novartis Crop Protection AG, Basel, Switzerland Novartis Crop Protection AG, Basel, Switzerland, Report No 953501 GLP Not Published Syngenta File N° CGA169374/1359	N	SYN
KIIIA 10.2.1/01	Volz, E	2004	Difenoconazole 250 EC formulation (A7402T): Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) in a 96-hour static test. [redacted] No. 853035 Not published GLP Syngenta File No. CGA169374/2554	N	SYN
KIIIA 10.2.1/02	Volz, E	2004	Difenoconazole 250 EC formulation (A7402T): Acute toxicity to *Daphnia magna* in a 48 hour immobilization test. RCC Ltd. Switzerland Report No. 853039 Not published GLP Syngenta File No. CGA169374/2552	N	SYN
KIIIA 10.2.1/03	Volz, E	2004	Difenoconazole 250 EC formulation (A7402T): A 72 hour algal growth inhibition test with *Scenedesmus subspicatus*. RCC Ltd. Switzerland Report No. 853037 Not published GLP Syngenta File No. CGA169374/2553	N	SYN

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KIIIA1 10.4.3/01	Decker, U.	1993	Testing for the toxicity of CGD 96430 F to foraging honey bees, (apis mellifera L.) under semi-field (tent) conditions Novartis Crop Protection AG, Basel, Switzerland RCC Ltd., Itingen, Switzerland, Report No 323436 GLP Not Published Syngenta File N° CGA169374/1272	N	SYN
KIIIA1 10.5.1/01	Kleiner, R.	2000a	Acute dose-response toxicity of CGA 169374 EC250 (A-7402 G) to the cereal aphid parasitoid Aphidius rhopalosiphi (Destefani-Perez) under laboratory conditions Novartis Crop Protection AG, Basel, Switzerland BioChem GmbH, Cunnersdorf, Germany, Report No 99 10 48 083 GLP Not Published Syngenta File N° CGA169374/2095	Y	SYN
KIIIA1 10.5.1/02	Nienstedt, K.M.	1999a	CGA 169374 EC 250 (A-7402 G): laboratory acute toxicity test with the parasitic wasp, Aphidius rhopalosiphi (Hymenoptera: aphidiidae) Novartis Crop Protection AG, Basel, Switzerland Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland, Report No 1047.035.270 GLP Not Published Syngenta File N° CGA169374/1963	Y	SYN
KIIIA1 10.5.1/05	Longley, M.	2001b	A semi-field study to evaluate the effects of fresh and aged residues of CGA 169374 EC250 (A7402 G) on the Parasitic Wasp Trichogramma cacoeciae (Hymenoptera, Trichogrammatidae) Syngenta Crop Protection AG, Basel, Switzerland Ecotox, Ltd., Tavistock, Devon, United Kingdom, Report No ER-00-KCB141 GLP Not Published Syngenta File N° CGA169374/2105	Y	SYN

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
KIIIA1 10.5.1/06	Kleiner, R.	2001	Acute dose-response toxicity of CGA 169374 EC 250 (A-7402 G) to the predatory mite Typhlodromus pyri (SCHEUTEN) under laboratory conditions Syngenta Crop Protection AG, Basel, Switzerland BioChem GmbH, Cunnersdorf, Germany, Report No 99 10 48 084 GLP Not Published Syngenta File N° CGA169374/2131	Y	SYN
KIIIA1 10.5.1/07	Nienstedt, K.M.	1999b	CGA 169374 EC 250 (A-7402 G): laboratory acute toxicity test with the predacious mite Typhlodromus pyri Scheuten (Acari: phytoseiidae) Novartis Crop Protection AG, Basel, Switzerland Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland, Report No 1047.035.268 GLP Not Published Syngenta File N° CGA169374/1978	N	SYN
KIIIA1 10.5.1/08	Grimm, C.	1999	Toxicity of CGA 169374 EC 250 (A-7402 G) to the predacious mite Typhlodromus pyri Scheuten (Acari: Phytoseiidae) under extended laboratory conditions Novartis Crop Protection AG, Basel, Switzerland, Report No 983929 GLP Not Published Syngenta File N° CGA169374/1981	N	SYN
KIIIA1 10.5.1/09	Kleiner, R.	2000b	The effects of A-7402 G (Bardos Neu, 250 g/l difenoconazole) on the green lacewing Chrysoperla carnea Steph. under laboratory conditions Novartis Crop Protection AG, Basel, Switzerland BioChem GmbH, Cunnersdorf, Germany, Report No 99 10 48 055 GLP Not Published Syngenta File N° CGA169374/2040	Y	SYN
KIIIA1 10.5.1/10	Walker, H.M.	2001	Difenoconazole: A Tier II laboratory study to determine the effects of a 250 g l-1 EC formulation (A 7402 G) on the green lacewing, Chrysoperla carnea (Neuroptera: Chrysopidae) Syngenta Crop Protection AG, Basel, Switzerland Ecotox, Ltd., Tavistock, Devon, United Kingdom, Report No ER-01-HMA430 GLP Not Published Syngenta File N° CGA169374/2205	Y	SYN

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KIIIA1 10.5.1/11	Engelhard, E.K.	1997b	CGA 169374 EC 250 (A-7402 G): semi-field toxicity test with the seven-spotted lady beetle, Coccinella septempunctata L. (Coleoptera: coccinellidae) Novartis Crop Protection AG, Basel, Switzerland Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland, Report No 97-198-1047 GLP Not Published Syngenta File N° CGA169374/1463	N	SYN
KIIIA1 10.5.1/12	Longley, M.	2001c	A Semi-field study to evaluate the effects of fresh & aged residued of CGA 169374 EC 250 (A 7402 G) on the Hoverfly, Episyrphus balteatus (Diptera, Syrphidae) Syngenta Crop Protection AG, Basel, Switzerland Ecotox, Ltd., Tavistock, Devon, United Kingdom, Report No ER-00-KCB139 GLP Not Published Syngenta File N° CGA169374/2132	Y	SYN
KIIIA1 10.5.1/13	Reber, B.	1999a	Toxicity of CGA 169374 EC 250 (A-7402 G) to the predator Orius laevigatus Fiber (Heteroptera: Anthocoridae) under extended laboratory conditions Novartis Crop Protection AG, Basel, Switzerland, Report No 983928 GLP Not Published Syngenta File N° CGA169374/1965	N	SYN
KIIIA1 10.5.1/14	Kleiner, R.	1999	The effects of A-7402 G (Bardos Neu, 250 g/l difenoconazole) on the spider Pardosa spp. under laboratory conditions Novartis Crop Protection AG, Basel, Switzerland BioChem GmbH, Cunnersdorf, Germany, Report No 99 10 48 054 GLP Not Published Syngenta File N° CGA169374/2041	N	SYN
KIIIA1 10.5.1/15	Reber, B.	1999b	Acute toxicity of CGA 169374 EC 250 (A-7402 G) to the predatory ground beetle Poecilus cupreus L. (Coleoptera: Carabidae) Novartis Crop Protection AG, Basel, Switzerland, Report No 993512 GLP Not Published Syngenta File N° CGA169374/1964	N	SYN

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
KIIIA1 10.5.1/16	Müther, J.	2000a	A field study to evaluate the effects of CGA 169374 EC 250 (A 7402 G) on Predatory mites in an apple orchard in Italy Syngenta Crop Protection AG, Basel, Switzerland GAB Biotechnologie GmbH, Niefern, Germany, Report No 20001049/I1-NFTp GLP Not Published Syngenta File N° CGA169374/2097	Y	SYN
KIIIA1 10.5.1/17	Müther, J.	2000b	A field study to evaluate the effects of CGA 169374 EC 250 (A 7402 G) on Predatory Mites in an apple orchard in Germany Syngenta Crop Protection AG, Basel, Switzerland GAB Biotechnologie GmbH, Niefern, Germany, Report No 20001049/G1-NFTp GLP Not Published Syngenta File N° CGA169374/2096	Y	SYN
KIIIA1 10.6.1.1/01	Thun, S.	1993	Acute Toxicity in Earthworm according to OECD 207 Test Article: "CGD 96430 F" Novartis Crop Protection AG, Basel, Switzerland IBR Forschungs GmbH, Hannover, Germany, Report No 80-91-0423-06-90 GLP Not Published Syngenta File N° CGA169374/0763	N	SYN
KIIIA1 10.6.1.2/01	Nienstedt, K.M.	2001	A chronic toxicity and reproduction test exposing the earthworm Eisenia fetida to CGA 169374 250 EC (A-7402 G) in OECD artificial soil Syngenta Crop Protection AG, Basel, Switzerland Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland, Report No 1047.100.631 GLP Not Published Syngenta File N° CGA169374/2140	Y	SYN
KIIIA 10.6.1.2/01	Friedrich, S	2003	CGA169374/azoxystrobin: Sublethal toxicity of a 125/200 g/L SC formulation (A13703G) to the earthworm *Eisenia fetida* in artificial soil. Biochem agrar GmbH, Germany (Syngenta Report No. 2033574) Not published GLP Syngenta File No. ICI5504/2144	N	SYN

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
KIIIA 10.6.1.2/02	Friedrich, S	2003	CGA169374/paclobutrazol: Sublethal toxicity of a 250/125 g/L SC formulation (A14049A) to the earthworm *Eisenia andrei*. Biochem agrar GmbH, Germany (Syngenta Report No. 2033722) Not published GLP Syngenta File No. PP333/0729	N	SYN
KIIIA1 10.7.1/01	Maas, G.	1990	Effects on soil microflora activity Novartis Crop Protection AG, Basel, Switzerland Labor für Bodenuntersuchungen UF, Braunschweig, Germany, Report No N/A Not GLP Not Published Syngenta File N° CGA169374/0767	N	SYN
KIIIA1 10.8/01	Wälder, L.	2000	Herbicide profiling test to evaluate the phytotoxicity of CGA 169374 250 EC (A-7402 G) to terrestrial non-target higher plants Novartis Crop Protection AG, Basel, Switzerland, Report No 15 GLP Not Published Syngenta File N° CGA169374/2029	N	SYN

**: Protection for 5 years claimed from date of decision concerning listing in Annex I - the study report has not been submitted any of the Member States in support of an application for authorization, or (though the study report has been submitted) has not been used any of the Member States as the basis for decision on the initial authorization, or to maintain a given authorization, of a plant protection product before the date of submission of the dossier to Rapporteur Member State.*

***: Owners' code identifications and names (SYN = Syngenta, TDMG = Triazole Derivative Metabolite Group)*

The notifier carried out a literature search on the 12th February 2004 using a number of databases. These were searched for references to difenoconazole and its triazole, triazole alanine and triazole acetic acid metabolites associated with several different keywords. A list of references found was included in Doc. M-II. The RMS did not expect any of these studies from the open literature to provide relevant information that would change the conclusions drawn from the material submitted and none of the studies were requested. In addition, Doc. L-II presented a list of references owned by Syngenta or Triazole Derivative Metabolite Group but not submitted. Justifications were provided for why they were not submitted. The RMS agreed to the justifications provided.