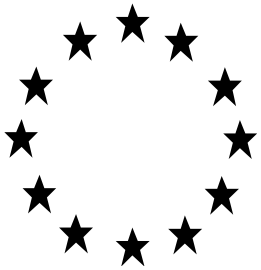
Draft Assessment Report



DIFENOCONAZOLE

Volume 3
Annex B.9
Ecotoxicology

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KEMI
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B.9 Ecotoxicology

ACTIVE INGREDIENT

Difenoconazole is a systemic triazole fungicide that controls a broad-spectrum of foliar, seed and soil-borne diseases, caused by Ascomycetes, Basidiomycetes and Deuteromycetes, in cereals, soya, rice, grapes, pome fruit, stone fruit, potatoes, sugar beet and several vegetable and ornamental crops. It is applied by foliar spray or seed treatment and acts by inhibiting ergosterol biosynthesis in fungal cell membranes thus preventing fungal development and penetration of the host crop. The ecotoxicological properties of difenoconazole active ingredient were evaluated in a series of laboratory studies summarized in this document.

DIVIDEND 030FS

The systemic, broad-spectrum fungicide 'Dividend' (A-9142 G) is a flowable concentrate containing 30 g/L difenoconazole. It is intended for use as a seed treatment to control a broad-spectrum of diseases in cereals. The Good Agricultural Practice (GAP) for the uses of the formulation in Northern and Southern Europe are listed in Table B.9-1. The main use of DIVIDEND 030FS is on winter wheat in Northern Europe with an equivalent maximum field rate of 12.3 g as/ha based on a seed planting rate of 205 kg seed/ha and a seed coating of 6 g as/100 kg seeds.

Table B.9-1: GAP for DIVIDEND 030FS (A-9142 G) in cereals in Northern and Southern Europe

	Northern Europe	Southern Europe
Product [mL/100 kg seed]	100 - 200	100 - 200
Difenoconazole [g/100 kg seed]	3.0 - 6.0	3.0 - 6.0
Seed planting rate depending on the cereal crop [kg seed/ha]	105 - 205	175 - 205
Equivalent field application rate: Difenoconazole [g as/ha]	6.3 – 12.3	5.3 – 12.3

Table B.9-2: Initial concentration of difenoconazole on seeds

Crop	Weight of 1000 grains (g)	Treatment rate (mg as/kg seeds)	Difenoconazole per seed (mg ai)
Wheat	50	60	0.003

SCORE 250EC

The systemic, broad-spectrum fungicide A-7402 T (Score® 250 EC) is an emulsifiable concentrate containing 250 g/L difenoconazole. It is intended for use as a foliar spray to control a broad-spectrum of diseases in pome fruit and vegetables. The proposed use patterns for critical uses in pome fruit and carrots in northern and southern Europe (NE, SE) are presented in Table B.9-3. The risk assessment will be based on exposure values estimated for the maximum use rate 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.

Table B.9-3: Critical use patterns of SCORE 250EC (A-7402 T) in Northern and Southern EU (N EU and S EU).

Crop	Application rate (g as/ha)	Number of applications	Application interval (days)	Growth stage at first application	Crop interception (%; from FOCUS groundwater)

Pome fruit (N EU)	56.25	4	7	Flowering	65
Pome fruit (S EU)	75	4	7	Flowering	65
Carrots (N EU and S EU)	125	3	14	BBCH 42/43*	80

**BBCH 1994. Compendium of growth stage indication keys for mono- and dicotyledonous plants – extended BBCH scale. Ed Strauss, R., Published by BBA, BSA, IGZ, IVA, AgrEvo, BASF, Bayer and Ciba.*

Where data for this formulation is required, data was presented from studies conducted with the formulations A-7402 G, A-7402 A, A-7402 F and A-7402 H, which are predecessors of the current A-7402 T formulation. Based on information on the compositions of the tested formulations provided by the notifier and presented in the confidential Annex C of this DAR, different amounts and kinds of solvents were used in the formulations. For two formulations (A-7402 F and A-7402 H), the used solvents are less toxic to tested aquatic organisms than the solvent used in the representative formulation. Therefore, studies on these formulations are not considered to cover the toxicity of the representative A-7402 T. However, in cases these formulations were used (aquatic organisms and non-target terrestrial arthropods), also tests with relevant formulations are available which are sufficient for the risk assessment and hence there is no need for further data.

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

B.9.1.1 Acute oral toxicity

ACTIVE INGREDIENT

Reference:	**Fletcher, D.W. (1988). 21-day acute oral LD_{50} study with CGA 169374 technical in mallard ducks. [redacted], USA. Unpublished report no. 86DD37. (Syngenta File No 169374/0014).**
Guideline:	OECD Guideline 205; US EPA FIFRA 71-2
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number FL 851406, purity 96.1%.
Species:	Mallard ducks (*Anas platyrhynchos*), 30 weeks old.
Treatments:	Technical difenoconazole prepared in corn oil was administered *via* oral intubation at doses of 0, 1470 and 2150 mg/kg.
Number of animals:	The test incorporated 5 male and 5 female birds per dose.
Duration:	21 days.
Test conditions:	Test temperature was 15 – 30C°, and the relative humidity 64 – 100%.
Observations:	Birds were monitored daily for mortality and the appearance of symptoms. Food consumption was recorded on days 3, 7, 14 and 21 while bodyweight was recorded on days 0, 3, 7, 14 and 21. On day 21, 2 female and 2 male birds were randomly selected from each dose group for gross pathological examination.
Data analysis:	Not stated, not needed.

Results:

No mortalities, abnormal behaviour, symptoms or abnormal tissue alterations were recorded during the study. Body weight and food consumption data are presented in Table B.9.1.1-1. The data indicates that treatment with difenoconazole did not have a significant effect on bodyweight. Birds exposed to 1470 and 2150 mg/kg did experience a depression in food consumption relative to untreated birds between days 1 and 3. However, this effect was transient as food consumption levels in treated birds between days 4 and 21 were similar to those observed in untreated birds.

Table B.9.1.1-1: Effect of difenoconazole on body weight and food consumption in mallard duck

Measurement	Day	Dose (mg/kg)		
		0	**1470**	**2150**
Mean bodyweight (g)	0	1084	1139	1131
	3	1142	1135	1133
	7	1124	1165	1172
	14	1152	1203	1160
	21	1176	1234	1195
Mean food consumption (g per bird per day)	1-3	100	75	83
	4-7	104	103	126
	8-14	123	129	131
	15-21	120	135	118

In the absence of significant effects on mortality, body weight or food consumption at any of the concentrations tested, the acute oral LD_{50} for difenoconazole in mallard duck was considered to be > 2150 mg/kg.

RMS comments:

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

Reference:	**Leopold, M.A. (1993). Acute oral toxicity study with CGA 169374 technical in Japanese Quail. [redacted] Unpublished report no. 104388. (Syngenta File No 169374/0843)**
Guideline:	US EPA FIFRA 71-1 (1989).
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number P.807002, purity 91.8%.
Species:	Japanese quail (*Coturnix coturnix japonica*), 11 weeks old.
Treatments:	Technical difenoconazole was administered orally in gelatine capsules at doses of 0, 125, 250, 500, 1000 and 2000 mg/kg.
Number of animals:	5 male and 5 female birds per dose.
Duration:	15 days
Test conditions:	Test temperature was 20 – 25 °C, relative humidity 40 – 80%.
Observations:	Birds were monitored daily for the appearance of symptoms and mortality. Food consumption was recorded on days 4, 8 and 15 while bodyweight was recorded on days 0, 1, 8 and 15. All surviving birds were sacrificed on day 15 for gross pathological examination.

Data analysis: Dunnett's t-test.

Results:

With the exception of 1 female treated with 2000 mg/kg, no mortalities were recorded during the study. The female treated with 2000 mg/kg suffered convulsions and was recumbent before being killed on day 2. Fluid faeces were recorded at all doses levels except control, within 24 hours of treatment. Lethargy was also recorded in some birds exposed to 250, 500, 1000 or 2000 mg/kg. However, both symptoms disappeared in surviving birds by day 3.

Body weight and food consumption data are presented in Table B.9.1.1-2. With the exception of birds exposed to 250 mg/kg, difenoconazole treatment did not significantly affect bodyweight gain over 15 days. Male and female birds dosed with 250 mg/kg suffered 76 and 77% reductions in weight gain relative to untreated birds. However, as this effect was not seen at higher doses, it was not considered a significant consequence of difenoconazole treatment. Food consumption during the first four days of the test was significantly reduced in female birds treated with 500 mg/kg and all birds treated with 1000 and 2000 mg/kg. However, consumption between days 4 and 15 was not significantly affected by difenoconazole treatment.

Post-mortem examination of the female bird from the 2000 mg/kg treatment group, killed on day 2, revealed haemorrhages and pale patches on the liver. Surviving birds examined on day 15 showed no pathological abnormalities.

Table B.9.1.1-2: Effect of difenoconazole on body weight and food consumption in Japanese quail.

Dose (mg/kg)	Gender	Mean bodyweight (g)			Mean bodyweight gain (g)	Mean food consumption (g per bird)		
		Day 1	Day 8	Day 15	Days 1-15	Days 1-4	Days 4-8	Days 8-15
0	M	170	191	188	17	84	108	151
	F	169	186	191	22	95	120	182
125	M	167	186	190	24	82	99	163
	F	166	183	183	17	86	103	146
250	M	167	184	171	4*	83	102	134
	F	170	185	175	5*	83	92	125
500	M	168	187	191	22	82	107	161
	F	168	179	184	16	74*	97	143
1000	M	167	183	185	18	56*	102	151
	F	171	187	191	20	71*	107	162
2000	M	165	171	183	16	40*	105	152
	F	167	174	184	17	34*	126	198

**significantly different from untreated control ($p \leq 0.05$)*

The acute oral LD_{50} of difenoconazole in Japanese quail was considered to be > 2000 mg as/kg.

RMS comments:

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

B.9.1.2 Short term dietary toxicity

ACTIVE INGREDIENT

Reference:	**Fletcher, D.W. (1988b). 11-day acute dietary LC50 study with CGA 169374 technical in mallard ducklings. [redacted] USA. Unpublished report no. 86DC69. (Syngenta File No 169374/0290)**
Guideline:	OECD Guideline 205; US EPA FIFRA 71-2.
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole batch number FL 851406, purity 96.1%
Species:	Mallard ducklings (*Anas platyrhynchos*).
Treatments:	Technical difenoconazole prepared in corn oil and mixed with feed at doses of 312, 625, 1250, 2500 and 5000 ppm, was fed to 8-day old ducklings for 5 consecutive days.
Number of animals:	5 control groups of 10 birds that were fed corn oil mixed with feed, and 1 group of 10 birds for each test concentration.
Duration:	After 5 days exposure, treated diets were removed and birds were fed untreated feed for a further 6 days.
Test conditions:	Test temperature was 24 – 30°C, relative humidity 71 – 91% and continuos light.
Observations:	Birds were monitored daily for mortality and the appearance of symptoms. In addition, birds were weighed on days 0, 8 and 11 while food consumption was recorded on days 5, 8 and 11. On day 11, 4 birds were randomly selected from each dose group for gross pathological examination.
Data analysis:	Not stated, not needed.

Results:

Measured concentrations in the diet ranged between 81 and 101% of the nominal values. Therefore the results are based on nominal concentrations.

Body weight, food consumption and mortality data are presented in Table B.9.1.2-1. One bird from each of the control, 1250 ppm and 2500 ppm treatments and three birds from the 5000 ppm treatment, died during the study. Exposure to difenoconazole concentrations up to 1250 ppm did not cause significant, consistent changes in bodyweight or food consumption relative to untreated birds. However, birds exposed to doses of 2500 and 5000 ppm suffered a 34% reduction in bodyweight by day 8, but regained weight to 85 and 125%, respectively, of their original bodyweight by day 11. However, birds exposed to doses of 2500 ppm consumed approximately 61% less food than control birds during the exposure phase and approximately 42% less during the 6-day recovery phase. Meanwhile, birds exposed to 5000 ppm consumed 62%, less food than control birds during the exposure phase and 77% less between days 5 and 8. These birds did regain their appetite between days 8 and 11 when they consumed only 16% less than control birds.

Gross pathological examination of birds dying during the study revealed hemorrhagic body cavities in those birds exposed to 1250 and 2500 ppm and in all but one bird exposed to 5000 ppm, and autolysis in two of the 5000 ppm birds. Examination of those birds sacrificed on day 11 did not reveal any visible tissue abnormalities.

Table B.9.1.2-1: Effect of difenoconazole on body weight, food consumption and mortality in mallard ducklings.

Dose (ppm)	Mean bodyweight (g)			Mean food consumption (g/bird/day).			Mortality (%)
	Day 0	Day 8	Day 11	Day 5	Day 6- 8	Day 9-11	Day 11
0	156	154	190	27	32	29	10
0	150	175	192	24	29	28	0
0	145	135	139	26	22	28	0
0	145	158	180	26	28	30	0
0	155	144	172	20	28	34	0
312	145	194	196	27	35	38	0
625	156	133	149	22	25	34	0
1250	156	171	217	24	25	45	10
2500	155	102	134	9	17	19	10
5000	156	102	195	9	7	35	30

As 50% mortality did not occur at any of the doses tested, the 5 day dietary LC_{50} of difenoconazole in mallard ducklings was considered to be >5000 ppm.

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.

Based on the food consumption data, it seems that an avoidance effect had occurred at the two highest test concentrations. However, at the concentrations expected at the proposed seed treatment rate of difenoconazole in cereals (60 mg as/kg seed) or on vegetation or other feed items in the field after the maximum spray application (0.125 kg as/ha), no avoidance is anticipated.

Since the results were only reported in dietary concentrations, a re-calculation to daily dose is needed in accordance with the guidance document SANCO 4145/2000. This was provided by the notifier in Document M-III. The calculation was based on the mean body weight on days 0 and 8 and the mean daily food consumption days 0-5 at the highest test concentration. The resulting daily dose LD_{50} value was >348.8 mg as/kg bw per day (highest dose tested). Since no records were made on possible spill of food during the test, the estimated daily doses may be over-estimated. However, the proposed value will be used for the risk assessment.

Reference:	**Fletcher, D.W. (1988c). 9-day acute dietary LC_{50} study with CGA 169374 technical in bobwhite quail. [redacted], USA. Unpublished report no. 87QC106. (Syngenta File No 169374/0332)**
Guideline:	OECD Guideline 205; US EPA FIFRA 71-2.
GLP:	Yes.

Material and methods:

Test substance:	Technical difenoconazole, batch number FL 861408, purity 95.2%.
Species:	Bobwhite quail (*Colinus virginianus*), 14-day old birds
Treatments:	Technical difenoconazole was prepared in corn oil and mixed with feed at doses of 312, 625,1250, 2500 and 5000 ppm.
Number of animals:	The test incorporated 5 control groups of 10 birds, and 1 group of 10 birds for each test concentration.
Duration:	5 days exposure, 4 consecutive days with untreated food.
Test conditions:	Test temperature was 32 - 39°C, relative humidity 28 – 34%, continuous light.
Observations:	Birds were monitored daily for mortality and the appearance of symptoms. In addition, birds were weighed on days 0 and 9 while food consumption was recorded on days 5 and 9. On day 9, 4 birds were randomly selected from each dose group for gross pathological examination.
Data analysis:	Not stated, not needed.

Results:

Measured concentrations in the diet ranged between 85 and 97% of the nominal values. Therefore the results are based on nominal concentrations.

Body weight, food consumption and mortality data are presented in Table 9.1.2-2. Birds exposed to difenoconazole doses of up to 1250 ppm did not exhibit signs of toxicity or abnormal behaviour throughout the test. Birds exposed to difenoconazole concentrations of 2500 and 5000 ppm appeared anorexic and lethargic within 3 days of test initiation and 60% of those receiving 5000 ppm died between days 3 and 6.
Exposure to difenoconazole doses of 312 and 625 ppm did not cause significant changes in bodyweight or food consumption relative to untreated birds. However, exposure to doses of 1250, 2500 and 5000 ppm caused reductions in bodyweight and food consumption of up to approximately 35% and 70%, respectively, relative to untreated birds.

Gross necropsy of those birds dying during the study showed autolysis in one bird. The remaining five and those sacrificed on day 9 did not show any tissue alterations.

Table B.9.1.2-2: Effect of difenoconazole on body weight, food consumption and mortality in bobwhite quail.

Dose (ppm)	**Mean bodyweight (g)**		**Mean food consumption (g/bird/day)**		**Mortality (%)**
	Day 0	Day 9	Days 0-5	Days 6-9	Day 9
0	33	60	9	9	0
0	33	58	7	8	0
0	33	58	7	8	0
0	33	61	7	9	0
0	33	62	8	9	0
312	33	61	7	8	0
625	33	57	7	9	0
1250	33	47	5	8	0
2500	33	44	4	7	0
5000	33	39	3	6	60

The 5 days dietary LC_{50} of difenoconazole in bobwhite quail was estimated to be 4760 ppm (95% confidence interval ($p \geq 0.05$) 4103 to 5522 ppm).

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.

Based on the food consumption data, it seems that an avoidance effect had occurred at the two or three highest test concentrations. However, at the concentrations expected at the proposed seed treatment rate of difenoconazole in cereals (60 mg as/kg seed) or on vegetation or other feed items in the field after the maximum spray application (0.125 kg as/ha), no avoidance is anticipated.

Since the results were only reported in dietary concentrations, a re-calculation to daily dose is needed in accordance with the guidance document SANCO 4145/2000. This was provided by the notifier in Document M-III. The calculation was based on the mean body weight on days 0 and 9 and the mean daily food consumption days 0-5. All test concentrations were converted to daily dietary dose values and the LD_{50} was estimated by non-linear interpolation. The resulting daily dose LD_{50} value was 392 mg as/kg bw per day. Since no records were made on possible spill of food during the test, the estimated daily doses may be over-estimated. However, the proposed value will be used for the risk assessment.

METABOLITES

Short-term dietary tests have also been conducted with the difenoconazole metabolite CGA 131013 (triazolyl alanine), which is formed in plants. The difenoconazole metabolite CGA 71019 is formed predominantly in soil and therefore dietary studies have not been conducted with this metabolite. However, CGA 71019 has been included in an avian chemosterilant screening programme carried out by the US Fish and Wildlife Service. These data are summarised in Section 9.1.3. of Annex B.

Reference:	**Beavers, J.B. (1983a). A dietary LC_{50} study in the mallard with CGA 131013 [redacted] Unpublished report no. 108-222. (Syngenta File No 131013/0034)**
Guideline:	US EPA FIFRA 71-2 (1982).
GLP:	No.
Material and methods:	
Test substance:	CGA 131013 (triazolylalanine), batch number TLB 1207, purity 97.5%.
Species:	Mallard duck (*Anas platyrhynchos*) , 10 days old.
Treatments:	The birds were offered diet containing a nominal concentration of 5000 ppm CGA 131013, dispersed in corn oil.
Number of animals:	A single group of 10 young birds (limit test). Five similar sized control groups were offered basal diet during the entire course of the study.

Exposure duration:	5 days exposure. Thereafter, birds received basal diet and were observed for a further 3 days.
Test conditions:	The environmental conditions during the study were as follows: temperature of 27 ± 15°C and a photoperiod of 14 hours of light per day. Relative humidity was not reported. Food and water were available *ad libitum*. Three days acclimatisation period in battery brooders (approximately 72 x 90 x 24 cm).
Observations:	All birds were observed daily during the study for mortality and symptoms of toxicity. Body weights were recorded on day 0 at the initiation of the study, on day 5 and at the termination of the study on day 8. Feed consumption was determined over days 0 to 5 and days 6 to 8.
Data analysis:	Not stated, not needed.

Results:

Mortality and symptoms of toxicity: There were no mortalities, no overt signs of toxicity nor behavioural abnormalities in the control groups and at 5000 ppm CGA 131013, the only concentration tested. Therefore, the LC_{50} was determined to be greater than 5000 ppm CGA 131013.

Food consumption and body weight: No effects on body weight or feed consumption were observed in the treatment group.

Table B.9.1.2-3: Effect of CGA 131013 on body weight, food consumption and mortality in mallard duck.

Dose (ppm)	Mean bodyweight (g)			Mean food consumption (g/bird/day)		Mortality (%)
	Day 0	Day 5	Day 8	Days 0-5	Days 6-8	Day 8
0	118	238	285	47	57	0
0	108	224	283	45	56	0
0	122	248	316	50	65	0
0	115	247	319	55	66	0
0	110	251	311	46	64	0
5000	125	255	311	51	66	0

As a concentration of 5000 ppm CGA 131013 did not cause any mortalities, the acute dietary LC_{50} in mallard duck was considered to be >5000 ppm.

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.

Since the results were only reported in dietary concentrations, a re-calculation to daily dose is needed in accordance with the guidance document SANCO 4145/2000. This was provided by the notifier in Document M-III. The calculation was based on the mean body weight on days 0 and 9 and the mean daily food consumption days 0-5. The resulting daily dose LD_{50} value was 1342 mg as/kg bw per day. Since no records were made on

possible spill of food during the test, the estimated daily doses may be over-estimated. However, the proposed value will be used for the risk assessment.

Reference:	**Beavers, J.B. (1983b). A dietary LC_{50} study in the bobwhite with CGA 131013. [redacted] Unpublished report no. 108-221. (Syngenta File No 131013/0033)**
Guideline:	US EPA FIFRA 71-2 (1982).
GLP:	No.
Material and methods:	
Test substance:	CGA 131013 (triazolylalanine), batch number TLB 1207, purity 97.5%.
Species:	Bobwhite quail (*Colinus virginianus)*, 12 days old.
Treatments:	The birds were offered diet containing a nominal concentration of 5000 ppm CGA 131013, dispersed in corn oil.
Number of animals:	A single group of 10 young birds (limit test). Five similar sized control groups were offered basal diet during the entire course of the study.
Exposure duration:	5 days exposure. Thereafter, birds received basal diet and were observed for a further 3 days.
Test conditions:	The environmental conditions during the study were as follows: temperature of 36 ± 15°C and a photoperiod of 14 hours of light per day. Relative humidity was not reported. Food and water were available *ad libitum*. 11 days acclimatisation period in battery brooders (approximately 72 x 90 x 23 cm).
Observations:	All birds were observed daily during the study for mortality and symptoms of toxicity. Body weights were recorded on day 0 at the initiation of the study, on day 5 and at the termination of the study on day 8. Feed consumption was determined over days 0 to 5 and days 6 to 8.
Data analysis:	Not stated, not needed.

Results:

Mortality and symptoms of toxicity: There were no mortalities in the control groups. Only lesions of nostril picking were noted on day 5 and 8. There was one mortality on day 3 at the dose level of 5000 ppm CGA 131013, but was not attributable to the treatment. It was considered to be likely caused by cannibalism. No overt signs of toxicity nor behavioural abnormalities were observed in all other birds of the treatment group. Therefore, the LC_{50} was determined to be >5000 ppm CGA 131013.

Food consumption and body weight: No effects on body weight or feed consumption were observed in the treatment group.

Table B.9.1.2-4: Effect of CGA 131013 on body weight, food consumption and mortality in bobwhite quail

Dose (ppm)	Mean bodyweight (g)			Mean food consumption (g/bird/day)		Mortality (%)
	Day 0	Day 5	Day 8	Days 0-5	Days 6-8	Day 8
0	22	30	37	5	6	0
0	23	34	43	7	8	0
0	24	38	47	9	10	0
0	21	32	40	6	9	0

Dose (ppm)	Mean bodyweight (g)			Mean food consumption (g/bird/day)		Mortality (%)
0	23	37	45	7	10	0
5000	21	36	44	8	8	10

As a concentration of 5000 ppm CGA 131013 did not cause 50% mortality, the acute dietary LC_{50} in bobwhite quail was considered to be >5000 ppm.

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.

Since the results were only reported in dietary concentrations, a re-calculation to daily dose is needed in accordance with the guidance document SANCO 4145/2000. This was provided by the notifier in Document M-III. The calculation was based on the mean body weight on days 0 and 9 and the mean daily food consumption days 0-5. The resulting daily dose LD_{50} value was 1404 mg as/kg bw per day. Since no records were made on possible spill of food during the test, the estimated daily doses may be over-estimated. However, the proposed value will be used for the risk assessment.

FORMULATED PRODUCTS

DIVIDEND 030FS

Notifier: In accordance with Directive 91/414/EC, acute ecotoxicity data for the formulations are not required, as results from mammalian testing with the formulation do not indicate that the formulation is significantly more toxic than the active ingredient (**Document MIII, Section 3**). Hence, the risk assessments for birds have been based on ecotoxicity data from studies with difenoconazole.

SCORE 250EC

Notifier: In accordance with Directive 91/414/EC, data for the formulation are not required, as results from mammal testing with the formulation do not indicate that the formulation is significantly more toxic than the active substance, (**Document M-III, Section 3**). In addition, birds are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by volatilisation. Therefore, where oral exposure is the main route of exposure, toxicity data for active substance is used in preference to data from tests with formulation material.

RMS comments:

According to Directive 91/414, a study on the formulation should be required also when TERa or TERst are between 10 and 100, if exposure to the formulation is not unlikely. At the use of difenoconazole in the seed treatment formulation Dividend the relevant TER values were 11 – 88, however the levels of co-formulants are

probably negligible at the time of sowing. Therefore, the RMS agrees with the notifier that a formulation study is not needed for this formulation.

At spray application with Score in pome fruit, TERa is above 100, while TERst is 93 and 61 for insectivores and herbivores, respectively when the TER is based on the lowest LC_{50} value from avian dietary studies with the active ingredient. Considering that this value was a higher than-value, the TERst are probably slightly under-estimated, and it is likely that at least the TERst for insectivores would exceed 100. Although this is a borderline case, the RMS considers that a formulation study is not needed.

B.9.1.3 Subchronic toxicity and reproduction

ACTIVE INGREDIENT

Reference:	**Pederson, C.A. (1990). CGA 169374 technical: toxicity and reproduction study in mallard ducks. [redacted] Unpublished report no. 88DR31. (Syngenta File No 169374/0015)**
Guideline:	US EPA FIFRA 71-4.
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number FL 881994, purity 91.1%.
Species:	Mallard ducks (*Anas platyrhynchos*), 34 week old.
Treatments:	The test substance in acetone was mixed with feed at doses of 25, 125 and 625 ppm.
Number of animals:	The test included 16 pairs (1 male and 1 female) of birds for each test concentration and 16 pairs for a control treatment where birds were given feed mixed with acetone.
Exposure duration:	126 days.
Test conditions:	Test temperature was 34 - 39°C, relative air humidity 25 - 35%. The birds were acclimated for 31 days before start of exposure period in the test cages ca 40x60x40 cm in size. After 6 weeks of exposure, egg laying was induced by weekly increases in light levels by 4 hours per day until a maximum daylength of 16 hours was reached. Food and water was provided ad libitum throughout the study.
Observations:	Throughout the study, birds were monitored daily for signs of toxicity while food consumption was monitored twice weekly. In addition, birds were weighed individually at test initiation and termination and twice weekly until egg-laying began. Gross pathological examinations were performed on those birds dying during the study and on 50% of surviving adults at test termination. Eggs were collected and candled daily during the egg production period. Numbers of cracked or broken eggs were also recorded. Eggs were then transferred to an incubator and, after 24 days, to hatching trays. The number of hatched, unhatched and infertile eggs was then recorded for each 7-day hatch period. Eggs were also examined for stage of embryo development and eggshell thickness. After hatching, F1 generation ducklings were monitored daily for 14 days. Duckling weights were

recorded on days 1 and 14 and gross pathological examinations of selected ducklings were made on day 14.

Data analysis: Analysis of variance, ANOVA.

Results:

Measured concentrations in the diet were 71 – 109% of the nominal values. The results were based on nominal test concentrations.

With the exception of one 25 ppm bird and one 625 ppm bird that died, birds did not show any symptoms of toxicity or behavioural abnormalities throughout the study. As the deaths were not correlated with dose, they were not considered to be a consequence of exposure to difenoconazole. Abnormal pathological findings were recorded for both birds and a further 25 ppm bird sacrificed on day 126. Data on body weight and food consumption of the parent birds are presented in the table below. No significant differences were observed between treated and control birds.

Table B.9.1.3-1: Body weight data (grams) for mallard ducks in the parent generation.

	Control		25 ppm		125 ppm		625 ppm	
Week	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Initiation	1167(±48)	1096(±89)	1165(±75)	1111(±91)	1198(±140)	1078(±99)	1135(±85)	1031(±80)
2	1228(±61)	1121(±104)	1235(±74)	1154(±68)	1240(±105)	1131(±123)	1196(±86)	1079(±88)
4	1236(±87)	1144(±118)	1261(±75)	1160(±71)	1241(±89)	1147(±119)	1206(±84)	1077(±80)
6	1235(±83)	1160(±133)	1246(±59)	1153(±67)	1247(±87)	1126(±105)	1193(±92)	1077(±72)
8	1227(±83)	1161(±132)	1236(±59)	1192(±99)	1252(±94)	1162(±116)	1213(±91)	1090(±93)
18	1241(±76)	1212(±119)	1277(±101)	1258(±110)	1277(±87)	1275(±157)	1227(±84)	1165(±98)

Table B.9.1.3-2: Food consumtion data (grams) for mallard ducks in the parent generation.

Week	**Control**	**25 ppm**	**125 ppm**	**625 ppm**
2	125(±15)	124(±14)	121(±17)	127(±15)
4	123(±16)	124(±14)	120(±17)	122(±15)
6	117(±15)	122(±15)	115(±18)	122(±15)
8	127(±12)	131(±18)	133(±14)	130(±15)
10	148(±13)	146(±13)	144(±13)	146(±15)
12	158(±7)	159(±5)	158(±6)	153(±15)
14	168(±13)	171(±11)	176(±9)	168(±15)
16	191(±18)	194(±21)	195(±22)	182(±15)
18	187(±15)	192(±18)	188(±17)	183(±15)

Egg production, viability and eggshell thickness data are presented in Tables B.9.1.3-3 and B.9.1.3-4. Numbers of eggs laid and set, viable embryos, normal hatchlings and 14-day old survivors were not significantly affected. However, significant reductions of up to 5.3% in eggshell thickness were seen following exposure to 125 and 625 ppm difenoconazole. As reduced shell thickness was not accompanied by an increase in the number of cracked or defective eggs, this effect was not considered biologically significant. In 2 out of 11 hatches, statistically significant reductions in the bodyweight of 1-day old ducklings were seen at the 125 ppm treatment levels, but not at the higher dose, and no significant effects were seen based on the mean body weights from all hatches (see table B.9.1.3-4). After 14 days, significant reductions (at 99% confidence level) were seen in 3

hatches at 125 ppm, but only in one hatch confirmed at the higher dose level. The effects were not correlated with dose or consistent over time, and were therefore not considered to be treatment related.

Table B.9.1.3-3: Effect of difenoconazole on egg production and viability in mallard duck.

Measurement	Dose (ppm)							
	Vehicle control		25		125		625	
	Total	% of eggs laid	Total	% of eggs laid	Total	% of eggs laid	Total	% of eggs laid
Eggs laid	700	-	658*	-	782	-	673	-
Eggs defective	37	5.3	29	4.4	29	3.7	37	5.5
Eggs set	614	87.7	578	87.8	693	88.6	581	86.3
Viable embryos	560	80.0	548	83.3	618	79.0	537	79.8
Live 15-20 day embryos	552	78.9	545	82.8	610	78.0	524	77.9
Normal hatchlings	455	65.0	450	68.4	503	64.3	407	60.5
14-day old survivors	448	64.0	431	65.5	489	62.5	394	58.5

**significantly different from the control.*

Table B.9.1.3-4: Effect of difenoconazole on eggshell thickness

Dose (ppm)	Mean eggshell thickness (mm)	Mean body weight of 1-day old hatchlings (g)	Mean body weight of 14-day old ducklings (g)
Control	0.38	36	157
25	0.39	37	153
125	0.37*	35	152
625	0.36*	35	161

**significantly different from the control.*

Based on the lack of observed adverse effects at the highest test concentration, the proposed NOEC from the study was 625 ppm.

RMS comments:

The number of eggs laid at the 25 ppm dose level was statistically lower than in the control. However, since no significant effects were seen at higher dose levels, this effect is not considered to be treatment related. This is also the case for the effects seen on 1- and 14 days old hatchling body weight. Measured concentrations in the highest dose level dropped below 80% of nominal in two samples (week 6 and week 12). However, since the mean measured at that dose level was 82% (>80%) of the nominal, according to the criteria in OECD test guidelines (no 206) it is considered acceptable to base the results on the nominal values.

Due to the slight but significant effects on egg shell thickness, the NOEC from this study is 25 ppm. However, since this effect did not cause any significant effects on the number of defective eggs, it is not considered to be an adverse effect, and therefore the NOAEC of 625 ppm will be used in the risk assessment.

Since the results were only reported in dietary concentrations, a re-calculation to daily dose is needed in accordance with the guidance document SANCO 4145/2000. This was provided by the notifier in Document M-

III. The calculation was based on the mean body weight (1141 g) and the mean daily food consumption (148 g) during the exposure period. The resulting daily dose NOAEL value was 81mg as/kg bw per day.

Reference:	**Frey, L.T., Martin, K.H., Beavers, J.B. and Jaber, M. (2000). Difenoconazole: A reproduction study with the northern bobwhite. [redacted] Unpublished report no. 108-427. (Syngenta File No 169374/2065).**
Guideline:	US EPA FIFRA 71-4.
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number WM806228, purity 94.3%.
Species:	Bobwhite quail (*Colinus virginianus*), 28 weeks old.
Treatments:	Dietary concentrations of 20, 100 and 500 ppm.
Number of animals:	16 pairs of birds (1 male and 1 female) for each test concentration and the untreated control.
Exposure duration:	20 weeks
Test conditions:	Test temperatures were 18.5±2.3°C for the adult birds, and 26.9±1°C for the hatchlings. Relative air humidity was 55±12% and 62±12%, respectively. At the beginning of week 8, egg laying was induced by increasing day-length from 8 to 17 hours.
Observations:	Throughout the study, birds were monitored daily for signs of toxicity and food consumption was recorded weekly. In addition, birds were weighed at test initiation and termination and during weeks 2, 4, 6 and 8. Gross pathological examinations were performed on those birds dying during the study and on surviving adults at test termination. Eggs were collected daily from the onset of egg production and candled to detect cracks or abnormalities. Randomly selected healthy eggs were used for eggshell thickness measurements while remaining normal eggs were transferred weekly to an incubator and, after 21 days, to hatching trays. The number of hatched, unhatched and non-viable eggs was then recorded for each 7-day hatch period. Eggs were also examined for stage of embryo development. After hatching, F1 generation chicks were weighed and monitored daily for 14 days. Chick weights were then recorded on day 14.

Results:

Measured concentrations were 108 – 110% of the nominal values. The results were based on the nominal test concentrations.

With the exception of one control bird and one 100 ppm bird that died, birds did not exhibit any symptoms of toxicity or abnormal behaviour throughout the study. Gross pathological examination showed abnormal findings for both birds dying during the study and several other birds surviving to test termination. However, as these

observations were not correlated with dose, they were not considered to be treatment related. Data on body weight of the parent birds are presented in the table below.

Table B.9.1.3-5: Body weight data (grams) for bobwhite quail in the parent generation.

	Control		25 ppm		125 ppm		625 ppm	
Week	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Initiation	200(±12)	202(±10)	200(±9)	201(±12)	199(±9)	202(±10)	199(±9)	202(±10)
2	201(±12)	202(±11)	201(±9)	201(±12)	199(±11)	201(±10)	198(±10)	201(±10)
4	201(±13)	202(±11)	201(±9)	202(±13)	201(±12)	202(±10)	201(±10)	202(±10)
6	203(±14)	203(±11)	204(±9)	203(±13)	206(±12)	206(±10)	205(±11)	206(±10)
8	205(±13)	208(±13)	207(±11)	207(±13)	205(±11)	206(±11)	206(±11)	206(±11)
Termination	212(±16)	250(±18)	215(±16)	249(±20)	214(±15)	250(±18)	213(±16)	250(±18)

Egg production, viability, eggshell thickness and chick bodyweight data are presented in Tables B.9.1.3-6 and B.9.1.3-7. Difenoconazole concentrations up to 500 ppm did not cause significant changes in adult bodyweight, food consumption or eggshell thickness relative to untreated birds. Doses of 20 and 100 ppm did not have any significant effects on egg production, embryo viability, numbers of 14-day old survivors or hatchling bodyweight. However, exposure at 500 ppm did reduce egg production and the number of 14-day old survivors relative to levels in control birds. This effect was not statistically significant based on data from all hens, but if two "non-productive" hens (with no eggs) were excluded, there was a statistically significant difference compared to the control. Therefore, this observation was considered to be treatment-related.

Significant reductions in 1-day old hatchling bodyweight (day 1) were also observed for those chicks exposed to 500 ppm difenoconazole. Chick bodyweights recorded on day 14 did not show any significant effect of exposure to 20, 100 or 500 ppm difenoconazole.

Table B.9.1.3-6: Effect of difenoconazole on egg production and viability in bobwhite quail.

Measurement	**Dose (ppm)**							
	Vehicle control		**20**		**100**		**500**	
	Total	**% of eggs laid**	**Total**	**% of eggs laid**	**Total**	**% of eggs laid**	**Total**	**% of eggs laid**
Eggs laid	739		848	-	790	-	604*	-
Eggs defective	24	3.2	38	4.5	19	2.4	2	0.3
Eggs set	644	87.1	734	86.6	694	87.8	531	87.9
Viable embryos	599	81.1	686	80.9	670	84.8	467	77.3
Live 3 week embryos	594	80.4	683	80.5	668	84.6	461	76.3
Normal hatchlings	576	77.9	647	76.3	638	80.8	439	72.7
14-day old survivors	546	73.9	613	72.3	603	76.3	406	67.2

**statistically different from the control if two non-productive hens are excluded.*

Table B.9.1.3-7: Effect of difenoconazole on eggshell thickness and chick bodyweight.

Dose (ppm)	**Eggshell thickness (mm)**	**Chick bodyweight**			
		Day 1		**Day 14**	
		Number	**Mean (g)**	**Number**	**Mean (g)**
Control	0.227 ± 0.016	575	7 ± 1	546	27 ± 2
20	0.231 ± 0.016	644	6 ± 1	613	27 ± 3
100	0.227 ± 0.021	638	6 ± 0	603	26 ± 3

500	0.223 ± 0.012	434	6 ± 1*	406	25 ± 3

**statistically significant compared to the control*

Based on observations of reduced egg production and 1-day old hatchling bodyweight following ingestion of 500 ppm difenoconazole by the parental generation, the NOEC was considered to be 100 ppm.

RMS comments:

The study was well performed and reported. Since the results were only reported in dietary concentrations, a re-calculation to daily dose is needed in accordance with the guidance document SANCO 4145/2000. This was provided by the notifier in Document M-III. The calculation was based on the mean body weight (208 g) and the mean daily food consumption (20.2 g) during the exposure period. The resulting daily dose NOAEL value was 9.75 mg as/kg bw per day. This value will be used in the risk assessment.

Reference:	**Schafer Jr. EW, Brunton EC, Schafer EC, Chavez G (1982). Effects of 77 chemicals on reproduction in male and female Coturnix quail. Ecotoxicology and Environmental Safety, 6: 149-156. (Syngenta File No 64250/2654)**
Guideline:	Not applicable, published paper.
GLP:	Not applicable.
Material and methods:	
Test substance:	CGA 71019 (1*H*-1, 2, 4, triazole, soil metabolite of difenoconazole)
Species:	*Coturnix* quail (*Coturnix coturnix*)
Treatments:	As part of an avian chemosterilant screening programme, male birds were orally dosed once with CGA 71019 at a rate of 316 mg/kg by gavage. Control birds were dosed with the solvent carrier 1,2- propanediol.
Number of animals:	Seven fertile male birds.
Exposure duration:	One dose by gavage.
Test conditions:	Not reported.
Observations:	Egg fertility of the female mates was observed for 30-35 days, after which period the male quail were sacrificed and their testes extracted and weighed. Compounds causing a 50% reduction from control fertility rates over the final 15 days of the test and a combined testes weight of ≤ 1.1 g at sacrifice, were considered to have chemosterilant effects.
Data analysis:	Analysis of variance, ANOVA.

Results:

Mortality and fertility data are presented in Table B.9.1.3-8. Exposure of quail to an oral dose of CGA 71019 of 316 mg/kg did not cause mortality in male quail and did not affect egg fertility recorded up to 35 days after treatment.

Table B.9.1.3-8: Effect of CGA 71019 on male mortality and egg fertility

Treatment	Male LD_{50} (mg/kg)	Fertility (% of eggs laid)		Male mortality	Testes weight (g)
		Days 1-35	Days 20-35		
Control (1,2-propanediol; 2080 mg/kg)	> 2080	92	91	0	2.854
CGA 71019 (316 mg/kg)	> 316	86	79	0	3.746

Oral exposure to a dose of CGA 71019 of 316 mg/kg bodyweight was not considered to have a chemosterilant effect on *Coturnix* quail based on the criteria of 40% effect.

RMS comments:

The test conditions were not reported, and the results were not statistically analysed. Further, since only male birds were exposed, the study cannot be used to exclude the potential for reproductive effects of CGA 71019. The study is of low value for the risk assessment.

B.9.1.4 Acceptance of bait, granules or treated seeds by birds

The formulation A-9142 G (DIVIDEND 030FS) is used as a seed dressing in cereals. The palatability and dietary toxicity of treated seeds to birds was investigated on rock dove.

Reference:	**Gallagher SP and Beavers JB (1999). A-9142 G – A test for avoidance of treated wheat seed with the rock dove (*Columbia livia*) [redacted] Unpublished report no 108-416. Study dates 23-31 August 1999 (Syngenta File No. CGA 169374/1947)**
Guideline:	Draft OECD guideline Test for avian avoidance of pesticide-treated seeds and baits; BBA guideline VI.25-1.
GLP:	Yes
Material and methods:	
Test substance:	A-9142 G containing 30.6 g/L CGA 169374. Batch number P.902001.
Species:	Rock dove (*Columba livia)*
Treatments:	Wheat seed treated with A-9142 G at a rate of 0.2 L/100 kg seed was offered to rock doves for a 6-hour period on each of three successive days. Birds were then given untreated seed for 2 hours.
Number of animals:	The test incorporated six replicates of two birds for the treatment and for the control, in which birds were offered untreated seed instead of treated seed.
Exposure duration:	Following the 3-day exposure period, birds were fed commercial bird-seed and observed for 6 days for toxicological responses.
Test conditions:	Temperature 23.6±1.0°C, relative humidity 77±17%.
Observations:	Bird bodyweights were recorded on days –6 and –1 (prior to initiation of the exposure phase), as well as on days 3 and 8.
Data analysis:	One-tailed Student's t-test.

Results

Birds from control and A-9142 G treatments did not suffer any mortalities or exhibit abnormal behaviour during the test. Bodyweight and food consumption data is presented in the tables below. Exposure of birds to A-9142 G-treated wheat seed did not have a significant effect on food consumption or bodyweight.

Table B.9.1.5-1: Effect of A-9142 G on bodyweight in the rock dove

Assessment time (day)	Bodyweight	
	Control	A-9142 G
Day –6	533	530
Day –1	531	525
Day 3	526	544
Day 8	546	541

Table B.9.1.5-2: Effect of A-9142 G on food consumption in the rock dove

Assessment time (day)	Control		A-9142 G	
	6 hour	2 hour	6 hours	2 hours
0	20.2	11.8	15.0	15.2
1	16.6	10.7	16.1	12.8
2	18.0	9.6	15.0	13.8
Mean	18.3	10.7	15.4	13.9

Exposure to wheat seed treated with A-9142 G at a rate of 2 L/kg (60 mg difenoconazole/kg seed) did not have a significant effect on food consumption or bodyweight in the rock dove.

RMS comments

The results indicate that DIVIDEND 030FS has no repellent effect on rock dove. Hence, in the risk assessment the avoidance factor will be set to 1.

B.9.1.5 Summary of the toxicity studies and risk assessment for birds

Studies were available on the active ingredient (acute oral, short term dietary and sub-chronic) and on the plant metabolite CGA 131013 (short term dietary). No studies on the formulations were submitted, since results from mammalian testing indicated that the formulations were not more toxic than the active ingredient.

Table B.9.1.5-1: Summary of toxicity endpoints from avian studies with difenoconazole

Species	Exposure duration	Dose range	Results*	Reference
Acute oral toxicity				
Active ingredient				
Anas platyrhynchos (Mallard duck)	acute	1470 – 2150 mg/kg bw	LD_{50}>2150 mg/kg bw	Fletcher (1988a)
Coturnix coturnix japonica (Japanese quail)	acute	125 – 2000 mg/kg bw	LD_{50}>2000 mg/kg/bw	Leopold (1993)
Short-term dietary toxicity				
Active ingredient				
Anas platyrhynchos (Mallard duck)	5 days	312 – 5000 ppm	LC_{50}>5000 ppm (>349 mg/kg bw day)	Fletcher (1988b)
Colinus virginianus (Bobwhite quail)	5 days	312 – 5000 ppm	LC_{50} 4760 ppm (392 mg/kg bw day)	Fletcher (1988c)
MetaboliteCGA 131013				

Anas platyrhynchos (Mallard duck)	5 days	5000 ppm	LC_{50} >5000 ppm (>1342 mg/kg bw day)	Beavers (1983a)
Colinus virginianus (Bobwhite quail)	5 days	5000 ppm	LC_{50} >5000 ppm (>1404 mg/kg bw day)	Beavers (1983b)
Sub-chronic toxicity and reproduction				
Active ingredient				
Anas platyrhynchos (Mallard duck)	18 weeks	25 – 625 ppm	NOEL 625 ppm (81 mg/kg bw day)	Pederson (1990)
Colinus virginianus (Bobwhite quail)	20 weeks	20 – 500 ppm	NOEL 100 mg/kg (9.8 mg/kg bw day)	Frey *et al* (2000)

**LD_{50} = median lethal dose (50% mortality); NOEL = no observed effect level*

For the major metabolite in plants, CGA 131013, only a short term dietary study was available. However, from these results there are no indications that the metabolite is more toxic than the parent compound. This was also supported by results from mammalian studies, and therefore no further data is considered necessary.

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

B.9.1.5.1 SEED TREATMENT WITH DIVIDEND 030FS

B.9.1.5.1.1 First tier risk assessment

A risk assessment for birds 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. 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.

As difenoconazole is a systemic seed-treatment, birds may be exposed to difenoconazole by direct consumption of treated seed or by eating the shoots of germinated wheat seedlings. Exposure *via* other routes such as dermal, consumption of insects and inhalation is considered to be negligible. Therefore, exposure *via* these routes will not be considered further.

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 **granivorous birds**. 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 notifier considers that the seed treatment is expected to dissipate rapidly by dissolution in rain, dew or soil water. Moreover, 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 **SANCO/4145/2000** for seed-treatment, i.e. small 15 g granivorous bird such as the linnet. 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 – no dehusking)
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 birds 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.

According to the notifier, investigations into the metabolism of difenoconazole in a range of plant species has demonstrated that up to 60% of measurable residues in foliage and grain may exist as the metabolite, triazolyl alanine (CGA 131013). Therefore, the maximum residue of CGA131013 in grain was assumed to be 60% of the value estimated for parent difenoconazole. However, data to support this assumption was unclear. Therefore, the RMS proposed 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 was taken into account). Available data only cover the short term dietary toxicity to birds. These data indicated that the metabolite is less toxic than difenoconazole. This was also the case in short and long term studies on mammals (see Annex B, section 6). In the absence of acute and long term effect data for the metabolite on birds, a reasonable worst case approach is proposed, assuming that the metabolite is of equal toxicity as the parent compound.

The acute and short-term risk of difenoconazole and CGA 131013 to birds following the consumption of DIVIDEND 030FS-treated seed was assessed for a standard granivorous bird, with a body weight of 15 g (FIR/bw 0.38), as proposed in SANCO 4145/2000.

As difenoconazole is systemic, **herbivorous birds** may be exposed by the consumption of residues in plant tissues grown from seeds treated with DIVIDEND 030FS. A significant proportion of active ingredient is considered likely to remain on the seed coat, be lost into soil or taken up into root tissue that would not be available for consumption by birds.

However, for the first tier risk assessments, it was assumed that shoots are consumed by a herbivorous bird. 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. TER values were calculated for the skylark (FIR/bw 1.06), as proposed by the notifier, and for a medium sized herbivorous bird (FIR/bw 0.76) as proposed by SANCO/4145/2000 for early growth stages of cereals.

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 birds 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. As indicated for exposure *via* consumption of seed treated with DIVIDEND 030FS the maximum residue of CGA131013 in wheat seedlings was assumed to be 100% of the value estimated for parent difenoconazole, with correction for molecular weight (factor 0.38).

According to 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 **secondary poisoning and biomagnification in terrestrial foodchains**. Therefore, the risk of difenoconazole (log P_{ow} of 4.4) following the proposed use of DIVIDEND 030FS to earthworm-eating, fish-eating and predatory birds has been assessed by the notifier.

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

$$\mathrm{TER} = \frac{\mathrm{NOEL\ (mg/kg)}}{\mathrm{PEC_{worm}\ (mg/kg)}^{(1)}\ \mathrm{x}\ 1.1^{(2)}}$$

(1) $PEC_{worm} = PEC_{soil} * BCF$ where $BCF = C_{worm}/C_{soil} = (0.84 + 0.01\ K_{ow}) / f_{oc} * K_{oc}$

(2) 1.1 is a constant used to convert the PEC_{worm} to a daily dose and is based on a 100g bird eating 113 g worms per day (Crocker *et al.* 2001).

The PEC_{worm} was calculated as indicated above and an additional calculation was performed using a BCF value obtained from an earthworm bioaccumulation study (Van der Kolk, 2001). The resulting TER values for worm-eating birds following use of DIVIDEND 030FS as a seed-treatment are presented in Table B.9.1.5-2. The RMS considered that the BCF from the earthworm study was unreliable, and therefore only the TER values based on the estimated BCF_{worm} are valid for the risk assessment.

With regard to the metabolites formed in soil, CGA 71019 (max ca 23%) has a Log P_{ow} of ca -0.6, 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 the toxicity to birds is available. However, given that the maximum PEC_{soil} is ca 1/10 of that for difenoconazole, the metabolite would need to be >300 times more toxic than the parent compound for causing a concern for secondary poisoning via earthworms. Hence, the RMS considers that no further data is necessary.

Table B.9.1.5-2: Long-term risk to birds from secondary poisoning occurring by feeding on earthworms after seed treatment with DIVIDEND 030FS. PECsoil values corrected based on RMS evaluation in Annex B.8.

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
NOEL mg as/kg/day	9.75	9.75
TER	164	554
TER trigger	5	5

(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} = 3760)
(3) estimated from earthworm bioaccumulation study (Van der Kolk, 2001), considered by RMS to be unreliable.

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

$$TER = \frac{NOEL\ (mg/kg)}{PEC_{fish}\ (mg/kg)^{(1)}\ \times\ 0.21^{(2)}}$$

(1) $PEC_{fish} = PEC_{water} * BCF$

(2) 0.21 is a constant used to convert the PEC_{fish} to a daily dose and is based on a 1000 g bird eating 206 g fish per day (Crocker *et al.* 2001).

The resulting TER values for fish-eating birds from the proposed uses of DIVIDEND 030FS are presented in the table below.

Table B.9.1.5-3: Long-term risk to birds from secondary poisoning by feeding on fish after seed treatment with DIVIDEND 030FS.

Parameter	Value
PECsw (µg as/L)[1]	0.69
BCF[2]	320
PEC_{fish} (mg as/kg)	0.22
NOEL (mg as/kg bw/day)	9.75
TER	211
TER trigger	5

[1] *maximum predicted difenoconazole concentration in water based on FOCUS Step 1.*
[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 Pow value compared to the parent, and assuming that the metabolite is not significantly more toxic than the parent (ca 400x), the risk for secondary poisoning via fish is considered to be low.

Results from adsorption, distribution, metabolism and excretion (ADME) studies indicate that difenoconazole has a low bioaccumulation potential, as the compound is extensively metabolised and almost completely eliminated within 7 days (**Document MIII, Section 3**). There will be negligible secondary exposure or bioaccumulation of difenoconazole, and a low risk to predatory birds is expected following use of DIVIDEND 030FS as a seed-treatment.

The risk for **exposure via drinking water** was assessed by RMS in accordance with SANCO/4145/2000 guidance. The concentration in drinking water that birds may be exposed to was considered to be equal to the PECsw. It was not considered that birds would be exposed in the field following seed treatment with difenoconazole.

Hence the PEC drinking water was assumed to be 0.69 µg as/L (Step 1, FOCUS calculation)and the total water ingestion rate for a small bird was calculated as $0.059*bw^{0.67}$ =0.0069 L/day. The daily dose of difenoconazole was calculated as PECdrinking water*total water ingestion rate/bw (0.00069*0.0069/0.01) which was compared to the long term NOEL of 9.8 mg as/kg bw day, resulting in a TER >20 000 which is above the Annex VI trigger and no further refinement is needed.

The calculated first tier TER values based on the assumptions described above are summarised in the table below.

Table B.9.1.5-4: First tier TER values for difenoconazole and the plant metabolite CGA 131013 after treatment of wheat seeds at a rate of 60 mg difenoconazole/kg seed.

Organism	Category	Time frame parent metabolite	FIR /bw (kg fw food/kg bw day)	C (mg as/kg)	MAF	f_{twa}	ETE (mg as/kg bw/d)	Tox value (mg as/kg bw/d)	TER
Granivorous bird	seeds	Acute	0.38	60	1	1	22.8	>2000	>88
		metabolite		23**	1	1	8.66	>2000*	>230
		Short-term	0.38	60	1	1	22.8	349	15
		metabolite		23**	1	1	8.66	>1342	155
		Long-term	0.38	60	1	0.33	7.60	9.8	**1.3**
		metabolite		23**	1	0.33	2.89	9.8*	**3.4**
Mediun sized herbivorous bird	young shoots	Acute	0.76	60	1	1	45.6	>2000	>44
		metabolite		23**	1	1	17.5	>2000*	110
		Short-term	0.76	60	1	0.50	22.8	349	15
		metabolite		23**	1	0.50	8.74	>1342	154
		Long-term	0.76	60	1	0.17	7.60	9.8	**1.3**
		metabolite		23**	1	0.17	2.97	9.8*	**3.3**
Small herbivorous bird (skylark)	young shoots	Acute	1.06	60	1	1	63.6	>2000	31.4
		metabolite		23**	1	1	24.4	>2000*	82
		Short-term	1.06	60	1	0.50	31.8	349	11
		metabolite		23**	1	0.50	12.2	>1342	110
		Long-term	1.06	60	1	0.17	10.8	9.8	**0.91**
		metabolite		23**	1	0.17	4.14	9.8*	**2.4**
Earthworm eating bird	earthworms	Long term	1.1	0.054	1	1	0.061	9.8	160
Fish-eating bird	fish	Long term	0.21	0.22	1	1	0.046	9.8	210

**metabolite assumed to be of equal toxicity as the parent compound*
***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 TER values for long term risk were below the Annex VI trigger of 5 for the active ingredient and the plant metabolite, and hence a refined risk assessment is needed. The risk to both granivorous and herbivorous birds needs to be addressed.

B.9.1.5.1.2 Refined long term assessment for birds following seed treatment with DIVIDEND 030FS.

In an additional submission in January 2006, the notifier has provided a refined risk assessment for granivorous and herbivorous birds at seed treatment with DIVIDEND 030FS. After discussions with the RMS, further proposals for the refinement were submitted in May 2006. A summary of the refined risk assessment is given below.

Granivorous birds

A small granivore, such as a linnet (used as a standard species in the first tier assessment), will dehusk cereal seeds before consumption and then a lot of the residue on the seed coat is removed. Using a generic dataset on measured seed residues, it has been shown that dehusking removes in the order of 85% of the residue on whole

seeds and therefore a dehusking factor of 0.15 has been recommended (**Edwards et al, 1998**[1]). Therefore, applying a dehusking factor of 0.15 to the small granivore risk assessment results in a TER_{LT} of 8.6, which is above the trigger value of 5 in Annex VI of 91/414.

However, there are other avian guilds which do not dehusk seeds so it is appropriate to consider which species are likely to feed upon cereal seeds and conduct a refined risk assessment for a range of relevant species. **Prosser & Hart (2005**[2]**)** recorded the bird species feeding on a range of crop seeds at feeding stations on UK farmland and counted numbers of seeds taken as well as proportion of dehusking. By reference to this paper, seven bird species have been selected on the basis of those with the highest numbers of visits for which wheat seed consumption was counted (as an indication of the most frequent visitors) and those with the highest numbers of seeds eaten (crow and pheasant). The two smallest species which did not dehusk seeds (dunnock and robin) were included in the risk assessment to ensure conservatism. The risk assessment is presented below using the lowest long-term NOEL of 9.75 mg as/kg bw/day for bobwhite quail, *Colinus virginianus*.

In May 2006, the notifier submitted an additional proposal to take into account dissipation from the treated seeds. Measured data was already available from a study submitted in January 2006 on systemicity of difenoconazole (**Bartlett, 2006**). The study is summarised and evaluated in the subsequent section on herbivorous birds. Recovery data from the treated seed is given in the table below.

Table B.9.1.5-5: Percentage recovery of radiolabelled difenoconazole from Dividend-treated wheat seed

Days after sowing	Total % recovery of radiolabelled difenoconazole from seed
0	100
2	53.1
6	23.4
9	21.16
14	6.39

These data were analysed by the notifier to generate a half-life for difenoconazole on wheat seed, using a non-linear, un-weighted least squares optimisation to fit single first order kinetics (also known as exponential decay). The fitting was carried out in a custom Microsoft Excel worksheet using the built-in Solver function to find the best fit. This analysis resulted in a DT_{50} of 3.1 days for difenoconazole on treated wheat seed. The model was considered a good fit to the data with an R^2 value of 0.988.
Therefore, to account for the dissipation of difenoconazole on the seed, using the measured DT_{50} of 3.1 days, a 7-day TWA residue can be calculated by applying an f_{TWA} of 0.505, based on the equation in the EC guidance document, SANCO/4145/2000. Results of the risk assessment based on this calculation are shown in the table below.

[1] *Edwards, P. J., Bembridge, J., Earl, M., Anderson, L and Jackson, D (1998): Estimation of Pesticide Residues on Weed Seeds for Wildlife Risk Assessment. SETAC Charlotte 1998. Abstract Book ref PMP036.*
[2] *Prosser P & Hart ADM (2005) Assessing potential exposure of birds to pesticide-treated seeds. Ecotoxicology,14: 679-691.*

Table B.9.1.5-6: Long-term risk assessment for birds feeding on DIVIDEND treated wheat seed.

Species	Bodyweight (g)	FIR (g)	C mg as/kg on seed	Factor to account for 1 week exposure	f_{twa} (dissipation from seed)	ETE (mg/kg)	DHF	TER
Chaffinch *Fringilla coelebs*	24	8.1	20	0.33	0.505	6.8	0.15	19
Yellowhammer *Emberiza citrinella*	31	9.7	20	0.33	0.505	6.3	0.15	21
Greenfinch *Carduelis chloris*	30	9.5	20	0.33	0.505	6.3	0.15	21
Robin *Erithacus rubecula*	18	6.6	20	0.33	0.505	7.3	1	**2.7**
Dunnock *Prunella modularis*	21	7.4	20	0.33	0.505	7.0	1	**2.8**
Crow *Corvus corone*	505	69	20	0.33	0.505	2.7	1	7.1
Pheasant *Phasianus colchicus*	1163	118	20	0.33	0.505	2.0	1	9.7

Following this refinement the TER for all species, with the exception of the robin and dunnock, exceed the trigger value of 5. Further consideration of the risk to robin and dunnock was therefore required and the notifier has submitted further data presented below to address the risk for these species.

Both the robin and dunnock are primarily insectivores which take some plant material, such as fruits and seeds, during the winter (**Cramp et al, 1977-94**[3]). For example, in farmland of southern Spain, between November–January, 22 stomachs of robins contained 26.0–42.3% (by volume, monthly averages) plant material, comprising entirely of berries and other pulpy fruit (**Herrera, 1977** as cited in **Cramp et al, 1977-94**). In contrast, during the breeding season in Crimea, stomach contents included only 0.7% plant material, with remainder comprising of invertebrates (**Kostin, 1983** as cited in **Cramp et al, 1977-94**). Studies of the diet of dunnock in England found no seeds in the diet April-July and an average of 52% by volume (predominantly weed seeds) of stomach contents over the whole year (**Cramp et al, 1977-94**). The field study by **Prosser & Hart (2005**[4]**)** which recorded wheat seed consumption by robin and dunnock in farmland was conducted during the winter and therefore can be considered as worst-case.

The data presented above, therefore, indicate that any potential exposure of robins and dunnocks would mostly be limited to the winter months. Furthermore, both species are predominantly birds of woodland and hedgerow rather than open field species and so, although they may forage around the edges of fields bordered by hedgerow or woodland, they would not be expected to occur in the large cereal fields without hedges found in Continental Europe. These findings are supported by the report from Central Science Laboratory (**Pascual et al, 1998**[5]) which, for the purposes of risk assessment, categorises both dunnock and robin as insectivores, mainly found in woodland/scrub and that when found in farmland feed mainly on the ground at the base of hedgerows and in field margins. This avoidance of open fields is also supported by the fact that both species were only recorded by

[3] *Cramp S et al eds. (1977 - 1994) The Birds of the Western Palearctic, Vols 1 – 9. Oxford: Oxford University Press.*
[4] *Prosser P & Hart ADM (2005) Assessing potential exposure of birds to pesticide-treated seeds. Ecotoxicology, 14: 679-691.*
[5] *Pascual J, Crocker J & Hart A (1998) Improving estimates of the exposure of non-target wildlife to pesticides in arable crops – a review of existing data - Discussion document for meeting on 15 May 1998. Central Science Laboratory; Project PN0910/0919 Milestone Report, May 1998. Available at: http://www.pesticides.gov.uk/approvals.asp?id=1183*

Prosser & Hart (2005) visiting baiting stations at the field edge (approximately 5m from the field edge), with no visits to baiting stations at least 50 m away from field boundaries. The notifier considered that the artificial feeding situation presented in the field study by **Prosser & Hart (2005)**, with a high density of seed in a small area, encouraged exploitation by species such as robin and dunnock that would not normally spend the significant periods of time foraging in bare, open arable fields that would be required to collect appreciable quantities of wheat seed following incomplete drilling. Under normal conditions (without the placement of surplus feed) these species would be expected to consume predominantly arthropods and weed seeds.

Based on the above information, indicating that robin and dunnock are only occasional field-edge foragers in fields bordered by hedgerows or woodland, that they feed predominantly on invertebrates only taking some seeds during the winter and the artificial feeding situation presented in the feeding study, the notifier proposed a PT value of 0.5 as a worst case. This would result in TER values of 5.2 and 5.4 for robin and dunnock, which are above the trigger value of 5 indicating that no further refinement is needed.

However, the information given above also indicates that robin and dunnock may not be the most relevant focal species to consider when assessing risk from DIVIDEND-treated wheat seed. A more appropriate small non-dehusking bird species to consider, that regularly forages in cereal fields and is a typical open field species, is the skylark, *Alauda arvensis.* Skylark is ranked by CSL (**Pascual et al, 1998**) as the highest priority farmland bird for risk assessment. A risk assessment for this species is presented below.

Table B.9.1.5-7: Long-term risk assessment for skylark feeding entirely on DIVIDEND-treated wheat seed

Species	Bodyweight (g)	FIR (g)	C mg ai/kg on seed	Factor to account for 1 week exposure	f_{TWA} to account for dissipation on seed	DHF	ETE (mg/kg)	TER
Skylark *Alauda arvensis*	38	10.8	60	0.33	0.505	1	2.84	3.4

The long-term TER for a skylark assuming that it feeds entirely on DIVIDEND-treated wheat seed is 3.4. However, the skylark is not entirely granivorous but is omnivorous, feeding on plant foliage and invertebrates as well as seeds. A representative diet for the skylark in spring is given below, taken from **Green (1978[6])** as cited by **Roelofs et al (2005[7])**.

Table B.9.1.5-8: Skylark diet in April - May (Green, 1978).

Food item	% wet weight in diet
Seeds	20
Leaves	50
Invertebrates	30

[6] Green, R.E. (1978) Factors affecting the diet of farmland skylarks, Alauda arvensis. Journal of Animal Ecology, 47, 913-928.

[7] *Roelofs, W.* et al. *(2005). Case Studies Part 2: Modelling the long-term risk of pesticides to individual breeding success and populations for birds and mammals.* **Ecotoxicology** *Vol 14, 8: 895 - 923.*

As a worst-case, leaves are assumed to comprise of grasses and cereal shoots, and seeds are all assumed to be cereal grain. The Daily Energy Expenditure (DEE) for the skylark was calculated based on the 'EC Guidance Document on Risk Assessment for Birds and Mammals under Council Directive 91/414/EEC', using the equation for "passerines" and assuming a body weight of 38 g.

$$\text{Log(DEE)} = \log(a) + b(\log bw)$$

Where log (a) = 1.0017 and log (b) = 0.7034

Then:

log (DEE) = 1.0017 + (0.7034 * log 38)

= 1.0017+ (0.7034*1.5798)

= 1.0017+1.1112

= 2.113

DEE = 129.7 kJ

Based on the DEE and energy content of food items consumed by the skylark (Appendix I, Table 3 of the 'EC Guidance Document on Risk Assessment for Birds and Mammals under Council Directive 91/414/EEC'), the daily consumption of the different diet components was calculated. Calculation steps and resulting data are shown in the table below.

Table B.9.1.5-9: Calculation of daily consumption of different diet components for the skylark

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 wet wt)	(kJ)	(g wet wt/day)
Grasses & cereal shoots	4.24	76	3.22	50	1.61		12.01
Cereal grain	14.48	80	11.58	20	2.32		4.80
Arthropods	6.46	76	4.91	30	1.50		7.20
Total	-	-	-	100	5.40	129.69	24.02

[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

Therefore, a skylark consuming a mixed diet, as given above, will consume 4.80 g of cereal grain per day, assuming that the entire seed content of its diet comprises cereal grain. Since DIVIDEND is applied as a seed treatment, it is reasonable to assume that while the treated seed is available the cereal shoots and arthropod components of the diet will contribute negligible residues of difenoconazole. A refined risk assessment for a skylark using the daily intake rate for cereal seeds, assuming a mixed diet, is given below. As above, it has been assumed that treated seed is available for the entire one week and that the DT_{50} of residues on seed is 3.1 days, resulting in an f_{TWA} of 0.505.

Table B.9.1.5-10: Refined long-term risk assessment for skylark feeding on DIVIDEND-treated wheat seed

Species	Bodyweight (g)	FIR (g)	C mg ai/kg on seed	Factor to account for 1 week exposure	f_{TWA} to account for dissipation on seed	DHF	ETE (mg/kg)	TER
Skylark *Alauda arvensis*	38	4.80	60	0.33	0.505	1	1.26	7.7

RMS comments on the refined long term assessment for granivorous birds

The RMS generally agrees with the risk assessment proposed by the notifier. It was noted however that the skylark diet proposed by the notifier was derived from measurements in spring in the study by Green (1978). Seemingly, the value used for proportion of cereal seeds in diet was an average of April (30%) and May (11%). Considering that in most parts of EU, sowing of cereals takes place in April, the RMS would propose to assume 30% cereal seeds in the diet as a worst case. This would result in a TER_{lt} of 5.1, ie. still slightly above the criteria, and no further refinement is needed.

Based on the proposed representative use, DIVIDEND is used also for autumn application in Northern EU. According to Green (1978) the proportion of cereal seeds in the diet of skylark is then much higher (56% in August, 70% in September, resulting in TER values of 2.7 and 2.2, respectively) compared to that in spring. However, it can be argued that the consumption of seeds in the autumn will consist to a significant extent of spilled grain being readily available on stubbles from harvesting operations. This spilled grain would not carry DIVIDEND residues and therefore, assuming that all of the cereal grain consumed in the autumn diet is newly-sown, DIVIDEND-treated grain would over-estimate exposure. Since there will be no spilled grain from harvesting available in the spring, the cereal grain component of the spring diet can only comprise newly-sown seeds. Therefore, using the spring diet gives a reasonable estimate of potential intake of DIVIDEND-treated seed by skylarks for both spring and winter cereals.

Further, at least in northern EU autumn applications are considered to take place outside the breeding season of wild birds, and therefore the risk for reproductive effects is considered unlikely. With regard to the breeding season of the skylark, this is reported by **Cramp et al (1977-94[8])** as extending from March to August in Europe. Hence, autumn applications would be acceptable in Southern as well as Northern EU.

Herbivorous birds

The notifier has provided additional data and a refined risk assessment for herbivorous birds. In order to assess the risk to herbivorous birds from consumption of cereal shoots emerging form Dividend-treated seeds it is necessary to have some estimate of movement of difenoconazole from the seed into the shoot. A laboratory study using radio-labelled difenoconazole has been performed by the notifier **(Bartlett, 2006)** which investigated the movement from wheat seeds into the shoots, and also a separate study **(Murfitt, 2006)** on seedling weights at different timings after emergence. The studies are summarised below.

8 Cramp S et al eds. (1977 - 1994) The Birds of the Western Palearctic, Vols 1 – 9. Oxford: Oxford University Press.

Report:	**Bartlett, D (2006) Radiolabel study to investigate systemicity of Difenoconazole from Dividend seed treatment. Jealotts Hill International, UK. Unpublished report no. FP 04 1001. (Syngenta File No 169374/2803)**
Guidelines:	Not applicable
Deviations:	Not applicable
GLP:	No

Methods: A solution of DIVIDEND was spiked with radiolabelled difenoconazole and applied to barley seeds at a rate of 36g as/100 kg seed. The seeds were laid on blue roll and treated with a micro-droplet applicator. Each seed was treated on one side with 5 microdroplets. On the side of the seed with the crease, the microdroplets were placed along the crease, while on the other side they were applied in a line down the middle of the seed. The droplets were left to dry for 2.5 hours before the seeds were carefully turned over and the other side treated. After a further 2 hours the seeds were planted. The treatment solutions were made up with the aim that each seed was treated with 10,000 dps. However when the treatment solution was checked it was found that each seed actually received an average of 3025 dps. For the 2-day sampling the seeds were placed in petri dishes containing damp filter paper. The rest of the seeds were planted in 3" pots of JIP 3 compost (52% sand, 28% silt, 20% clay, 9.4% organic matter, pH 5.9), at the rate of one seed per pot, and placed in the cool bay of the glasshouse (set at 18ºC day and 12ºC night).

At 2, 6, 9, and 14 days after sowing, the plants were sampled. At the earlier time points the seeds were shaken in 0.5ml of acetone to remove the unabsorbed chemical residues and this was quantified (seed surface) by liquid scintillation counting (LSC). The rest of the plant was separated into the various leaves and seed and combusted to determine the movement of the radiolabel. For the first few samplings the roots were also combusted. Eight seeds were treated for each time point, of which four were combusted.

Results

The mean percent recovery of radioactivity is summarised in the table below. The recovery figures are given as % of the initial amount per seed based on measured concentrations in the application solution, 3025 dps.

Table B.9.1.5-11: Percent recovery of radioactivity in wheat seedlings following seed treatment at a rate of 360 mg/kg seed. Mean of triplicate samples.

Days after sowing	seed surface (%)	seed (%)	leaf 1 (%)	leaf 2 (%)	leaf 3 (%)	root (%)	total recovery
0	not reported	not reported	not reported	not reported	not reported	not reported	100%
2	31.7	21.4	-	-	-	-	53.1
6	10.8	12.6	1.2	-	-	2.3	26.9
9	7.96	13.2	0.38	0.42	-	0.38	22.3
14	2.49	3.9	0.06	0.06	0.04	0.7	7.25

The highest recovery of radio-label from shoots was at 6 days after treatment (1 leaf stage) when 1.2% of radioactivity applied was recovered in the shoot.

RMS comments

The nominal treatment dose in this study was 6 times higher than the representative 60 mg as/kg seed, and it could be argued that the uptake in the shoot may not be lineary related to the concentration in the seed. However, since the recovery in the treatment solution (estimated to 3025 dps per seed) and on the seeds on day 2 after sowing (1606 dps) was approximately 1/3 and 1/6 of the nominal treatment rate, respectively (10,000 dps), the RMS considers that the study can be regarded as representative for the intended seed treatment use of difenoconazole. The maximum levels of radioactivity observed in the shoots, accounting for 1.2% of the "initial" dose will be used in the risk assessment as proposed by the notifier.

Report:	**Murfitt (2006): Investigation of crop seedling shoot weights at different timings after emergence.**
Guidelines:	Not applicable
Deviations:	Not applicable
GLP:	No
Methods:	A study was carried out to record weights of shoots of some major crops at various timings after emergence. Eight crops were investigated – wheat, barley, sunflower, oilseed rape, pea, sorghum, sugar beet and maize. Ten seeds of each crop were sown into compost in 8 cm diameter pots, one crop per pot. The compost comprised a mixture of 67% moss peat, 25% loam and 8% grit. The warm-climate crops, maize and sorghum, were grown in a glasshouse set to maintain at least 16ºC at night and 24ºC during the day. The remaining temperate crops were grown in a glasshouse set to maintain at least 12ºC at night and 16ºC during the night. All crops were sown on 18th November 2002. Daylength was maintained at 16 hours using supplemental lighting. Pots were watered manually onto the surface of the compost to ensure that the compost stayed moist. Four pots were sown for each crop, one to be harvested at each of 4 intervals after seedling emergence. Shoots were harvested at 1, 3, 7 and 14 days after emergence. The number of emergent shoots and total shoot weight for each pot was recorded and then a mean shoot weight calculated.

Results

A summary of the results (from wheat only, since this was the representative crop) is given in the table below.

Table B.9.1.5-12: Weights of wheat seedlings at various dates after emergence

	Days after emergence (25/11/2002)			
	+ 1 day	**+ 3 days**	**+ 7days**	**+ 14 days**
Recording date	26/11/2002	28/11/2002	02/12/2002	09/12/2002
No. of shoots emerged	7	10	8	9
Total Weight	0.083g	0.367g	1.163g	4.624g
Mean wt/shoot	0.012g	0.037g	0.145g	0.514g

RMS comments

The conditions used in this study are regarded as realistic for sowing of treated wheat seeds.

Since shoots at 1 and 2 days after sowing will not yet have emerged from the ground, the notifier proposed to use wheat shoots at 7 days after sowing when the shoot to seed weight ratio is 3.6 (seed weight 0.04 g). However, the RMS instead proposes to use the shoot weight data for estimation of a "time weighted average" shoot weight, in order to derive an f_{twa} based on dilution rate in the emerging shoots. Based on the shoot weights on days +1, +3, +7 and +14, and assuming a "first order" growth rate, the dilution DT_{50} would be 2.5 days, which would result in an f_{twa} of 0.17 over a 21 days time window. This value will be used in the RMS assessment.

RMS comments on the refined long term assessment for herbivorous birds

The RMS generally agrees with the proposed approach for risk assessment for herbivorous birds provided by the notifier. Regarding the shoot weight data, a time weighted average approach is proposed by the RMS, compared to the point estimate on day 7 as proposed by the notifier. The refined long term risk assessment for herbivorous birds 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. Calculations based on the RMS assumptions are given in the table below.

Table B.9.1.5-13: Refined long term TER values for herbivorous birds 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
Mediun sized herbivorous bird	young shoots	Long-term	0.76	0.72	1	0.17	0.093	9.8	105
		metabolite	0.76	0.27	1	0.17	0.034	9.8**	287
Small herbivorous bird (skylark)	young shoots	Long-term	1.06	0.72	1	0.17	0.13	9.8	75
		metabolite	1.06	0.27	1	0.17	0.049	9.8**	200

**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.*
***equal toxicity as for the parent compound was assumed.*

The refined long term TER values for herbivorous birds 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.9.1.5.2 SPRAY APPLICATION WITH SCORE 250EC

B.9.1.5.2.1 First tier risk assessment

A risk assessment for birds at dietary exposure following spray application of difenoconazole to pome fruit and carrots using the formulation SCORE 250EC was provided by the notifier in Document M-III, section 6.10.

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 first tier risk assessment is given below.

The Estimated Theoretical Exposure (ETE) values to difenoconazole for appropriate scenarios were estimated according to SANCO/4145/2000, based on the maximum use rate 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. ETE values were calculated using the following equation:

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

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 short and long-term risk assessments. For assessing acute exposure, special MAF_{90Fl} values were used as given in the Guidance Document. For assessing short-term and long-term exposure, MAF and f_{TWA} values were calculated from the following equations:

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

where n = number of applications
$k = \ln 2/DT_{50}$
i = interval between applications
t = averaging time

A measured foliar half-life of 7.7 days, representing 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 default f_{TWA} and MAF in plant material. As recommended in the Guidance Document, an averaging time equivalent to the application interval (7 and 14 days for orchards and carrots, respectively) was used to calculate f_{TWA} in order to ensure that the maximum TWA residue was not underestimated.

The first tier calculations were based on the standardised realistic worst-case scenarios recommended in the Guidance Document for orchard crops, i.e. small insectivorous bird consuming small insects (FIR/bw 1.04), and

for leafy crops, i.e. medium herbivorous bird (FIR/bw 0.76) consuming leafy crops and small insectivorous bird consuming small insects (FIR/bw 1.04). In the case of insects little is known on time-course of contamination and degradation. However, repeated applications are not expected to cause appreciable accumulation of residues, at least in foliage-dwelling insects, particularly as replacement of individuals due to migration and reproduction will contribute to the residue decline in the population. Therefore, Multiple Application (MAF) and time-weighted average factors (f_{TWA}) were not applied for residues in insects.

For the metabolite, CGA 131013, 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).

No long term assessment was provided for the metabolite, and no long term effect data are available. Based on the lower toxicity in the available short term dietary test (a factor ca 0.3 less toxic compared to the parent), and on the significantly lower short and long term toxicity for mammals, it is considered likely that the metabolite is not more toxic to bird reproduction than difenoconazole. The notifier stated that studies in mammals have shown low toxicity from CGA131013, with acute LD_{50}'s in both rats and mice of >5000 mg as/kg food and a lowest NOAEL of 100 mg as/kg bw/day in reproductive toxicity studies in rats. Again toxicity of the metabolite is significantly lower than for the parent, difenoconazole, which has an acute LD_{50} of 1453 mg as/kg bw/day and long-term NOAEL of 17.3 mg as/kg bw/day in rat. In the RMS calculations below, where avian data was missing it was assumed that the metabolite was of equal toxicity as the parent compound.

In accordance with SANCO/4145/2000, substances with a log P_{ow} greater than 3 have potential for bioaccumulation and should be assessed for the risk of **secondary poisoning and biomagnification in terrestrial food chains**. Therefore, the risk of difenoconazole (log P_{ow} of 4.4) following the proposed use of SCORE 250EC to earthworm-eating, fish-eating and predatory birds was assessed by the notifier.

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

$$TER = \frac{NOEL\ (mg/kg\ bw/day)}{PEC_{worm}\ (mg/kg)^{(1)}\ x\ 1.1^{(2)}}$$

(1) $PEC_{worm} = PEC_{soil} * BCF$ where $BCF = C_{worm}/C_{soil} = (0.84 + 0.01\ K_{ow}) / f_{oc} * K_{oc}$

(2) 1.1 is a constant used to convert the PEC_{worm} to a daily dose and is based on a 100g bird eating 113 g worms per day (Crocker *et al.* 2001).

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. The study on bioaccumulation in earthworms was not considered by RMS to be valid (see section B.9.2), and therefore the assessment should be based on the calculated BCF value (TER=20 and 28 in pome fruit and carrots, respectively). Otherwise, the RMS agrees with the assessment provided by the notifier. With regard to the metabolites formed in soil, CGA 71019 (max ca 23%) has a Log P_{ow} of ca -0.6, 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 the toxicity to birds is available. However, given that the maximum PECsoil is ca 1/10 of that for difenoconazole, the metabolite would need to be 40 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.9.1.5-14: Long-term risk to birds from secondary poisoning occurring by feeding on earthworms after spray application with SCORE 250EC. PEC_{soil} values corrected according to RMS evaluation in Annex B.8.

Parameter	Pome fruit (4 x 75g as/ha)		Carrots (3 x 125 g as/ha)	
	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)	9.75	9.75	9.75	9.75
TER	21	72	30	101

[1] maximum predicted difenoconazole concentration in soil following final application
*(2) BCF = Cworm/Csoil = (0.84 + 0.01 Kow) / foc * Koc (Kow =25118; foc =0.02; Koc =3760)*
[3] estimated from earthworm bioaccumulation study (Van der Kolk, 2001), assessed as unreliable by RMS

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

$$TER = \frac{NOEL\ (mg/kg\ bw/day)}{PEC_{fish}\ (mg/kg)^{(1)} \times 0.21^{(2)}}$$

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

[2] 0.21 is a constant used to convert the PEC_{fish} to a daily dose and is based on a 1000 g bird eating 206 g fish per day (Crocker *et al.* 2001).

The resulting TER values are presented in the table below. The resulting TER values are greater than the long-term trigger value of 5, indicating a low risk to fish-eating birds from the proposed uses of SCORE 250EC. 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 50x), the risk for secondary poisoning via fish is considered to be low.

Table B.9.1.5-15: Long-term risk to birds from secondary poisoning by feeding on fish after spray application with SCORE 250EC.

Parameter	Pome fruit	Carrots
PEC_{sw} (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/bw)	9.75	9.75
TER	34	53

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

The notifier stated that results from adsorption, distribution, metabolism and excretion (ADME) studies indicate that difenoconazole has a low bioaccumulation potential, as the compound is extensively metabolised and almost completely eliminated within 7 days (**Document M-III, Section 3**). Thus, there will be low secondary exposure and bioaccumulation of difenoconazole, and a low risk to predatory birds is expected following the proposed uses of SCORE 250EC.

The risk for exposure via drinking water was assessed by RMS in accordance with the SANCO/4145/2000 guidance. The concentration in drinking water that birds may be exposed to was considered to be equal to the PECsw. It was not considered that birds 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 maximum PEC drinking water was assumed to be 32 µg as/L (Step 1, FOCUS calculation for pome fruit)and the total water ingestion rate for a small bird was calculated as $0.059*bw^{0.67}$ =0.0069 L/day. The daily dose of difenoconazole was calculated as PECdrinking water*total water ingestion rate/bw (0.032*0.0069/0.01) which was compared to the long term NOEL of 9.8 mg as/kg bw day, resulting in a TER of 442 which is above the Annex VI trigger, and no further refinement is needed.

A summary of the assumptions made in the first tier risk assessment and the resulting ETE and TER values are listed in the table below.

Table B.9.1.5-16: First tier risk assessment for birds at the representative use of difenoconazole in carrots and pome fruit. Spray application with SCORE 250EC. Calculations for parent and plant metabolite CGA 131013.

Use pattern Organism	Category	Timeframe parent metabolite	FIR /bw (kg fw food/kg bw day)	RUD	MAF	f_{twa}	ETE	Tox value (mg as/kg bw/d)	TER
Carrots, 3 x 125 g as/ha, 14 days intervall.									
Medium sized herbivorous bird	leafy crops	Acute	0.76	87	1.3	1	10.74	>2000	>186
		metabolite		33**			4.08	>2000*	>490
		Short-term	0.76	40	1.5	1	5.70	349	61
		metabolite		15**			2.17	>1342	>618
		Long-term	0.76	40	1.5	0.64	3.65	9.8	**2.7**
		metabolite		15			1.15	9.8*	8.5

Insectivorous bird	small insects	Acute	1.04	52	1	1	6.76	>2000	>296
		Short term	1.04	29	1	1	3.77	349	93
		Long term	1.04	29	1	1	3.77	9.8	**2.6**
Earthworm eater	earthworms	Long term	1.1	C=0.32	-	-	0.36	9.8	28
Fish eater	fish	Long term	0.21	C=0.87	-	-	0.18	9.8	53
Pome fruit, Northern EU, 4 x 56.25 g as/ha, 7 days interval.									
Insectivorous bird	small insects	Acute	1.04	52	1	1	3.04	>2000	658
		Short term	1.04	29	1	1	1.70	349	205
		Long term	1.04	29	1	1	1.70	9.8	5.8
Pome fruit, Southern EU, 4 x 75 g as/ha, 7 days interval.									
Insectivorous bird	small insects	Acute	1.04	52	1	1	4.06	>2000	493
		Short term	1.04	29	1	1	2.26	349	154
		Long term	1.04	29	1	1	2.26	9.8	**4.3**
Earthworm eater	earthworms	Long term	1,1	C=0.45	-	-	0.50	9.8	20
Fish eater	fish	Long term	0.21	C=1.87	-	-	0.39	9.8	25

**assumed that the metabolite is equally toxic as the parent*
***RUD assuming that themetabolite accounts for 100% of the parent, with correction for molecular weight by a factor of 0.38.*

In the first tier calculations, all acute and short term TER values were above the trigger of 10 for both parent and the plant metabolite, indicating that no further refinement is needed. However, the long term TER values were below the trigger of 5 for herbivores (both parent and metabolite) and insectivors in carrots (parent), and for insectivors in pome fruit (parent) in Southern EU. Hence, refinement was needed.

B.9.1.5.2.2 Refined long term assessment for birds at the use of SCORE 250EC in carrots and pome fruit

The TER_{LT} values for difenoconazole were below the trigger value of 5, indicating that further assessment is required. The values were based upon the bird eating 100% treated insects or leafy crops over a long period (21 days) i.e. proportion of different food types in the diet PD = 1 and proportion of diet obtained in treated areas PT = 1. The notifier stated that these assumptions are highly unlikely considering the mobility of both birds and insects, and the short life-cycle of many small insects. Data from **Crocker *et al.* (1998)** indicates that 95% of blue tits spend less than 61% of potential foraging time among orchard trees. Taking foraging time to be 61%, a PT of 0.61 was applied to the insectivorous bird in pome fruit scenario for calculating a more realistic ETE and so refining the TER_{LT}. Taking into account also that the exposure via small insects is based on estimated initial residues without declination (eg due to migration and reproduction), it is likely that the risk for insectivorous birds would be low. It was noted that with regard to insectivorous birds in orchards PT<0.86 would be sufficient to result in a TER above the trigger value of 5. The RMS considers that the risk for insectivorous birds is sufficiently addressed.

With respect to the scenarios in carrot crops, again the notifier states that it is highly unlikely that birds will spend all of their time foraging in treated areas. Carrots are grown on a relatively small area of farmland. For example, in the UK (Europes' largest producer of carrots) in 2000 only 0.14% of arable land (FAOSTAT database) was used to grow carrots. This, coupled with the fact that carrots are generally grown on at least a five-

year rotation[9], will ensure that there are alternative crops other than carrot for foraging available in the vicinity for farmland birds. Further, the first application to carrots is not until BBCH 42/43 (late) so the foliage would not be expected to be at its' most palatable to herbivores. Hence it was considered reasonable to apply a PT of 0.5 for birds feeding in carrots. Although this was not supported by data, the RMS still considers the argumentation reasonable. However, the acceptability of this approach needs to be further discussed by MS. The revised TER_{LT} values for difenoconazole are given in the table below.

Table B.9.1.5-17: Refined long-term risk (TER_{LT}) to birds from exposure to difenoconazole residues at spray application with SCORE 250EC. Calculations by the notifier.

Crop use	Avian guild	Diet	PT	Endpoint value (mg/kg/day)	ETE (mg/kg/day)	TER_{LT}
Pome fruit	Small insectivore	Small insects	0.61	9.8	1.38	7.1
Carrot	Small insectivore	Small insects	0.5	9.8	1.88	5.2
Carrot	Medium herbivore	Leafy crops	0.5	9.8	1.47	6.6

B.9.1.5.3 RMS overall conclusion on the risk for birds 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 further refinement is needed.

In the long term assessment, a risk was identified for granivorous birds following seed treatment with DIVIDEND 030FS in the first tier assessment, but based on additional data and proposed refinements all TER values were above the trigger values and no further refinements are considered as necessary. Regarding spray applications with SCORE 250EC, discussions are needed on the acceptability of the proposed refinements of the risk assessment for small insectivores in pome fruit orchards and carrot cultivations and for herbivorous birds in carrot cultivations.

B.9.2 Effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2)

B.9.2.1 Acute toxicity to fish

ACTIVE INGREDIENT

Reference:	**Surprenant, D.C. (1987a). Acute toxicity of CGA 169374 to Rainbow trout (*Salmo gairdneri*) [redacted]. Unpublished report no. 87-6-2207. (Syngenta File No 169374/0017)**
Guideline:	US EPA FIFRA 72-1.
GLP:	Yes
Material and methods:	
Test substance:	Technical difenoconazole, batch number not stated, purity 96%.
Species:	Rainbow trout (*Salmo gairdneri*)
Treatments:	Aliquots of a difenoconazole stock solution prepared in dimethylformamide, were

[9] *Primrose McConnell's The Agricultural Notebook. 18th edition (1988). Eds RJ Halley and Soffe RJ. Butterworths, Sevenoaks, Kent.*

	introduced into tanks to produce 6 exposure concentrations (0.4, 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L) in a maximum final dimethylformamide concentration of 0.5 mL/L. The test incorporated one tank for each exposure concentration, one control tank prepared with 0.5 mL/L dimethylformamide and one control tank.
Number of animals:	Ten fish (mean weight 0.78 g; mean length 44 mm) were introduced into each tank.
Duration:	96 hours, static test
Test conditions:	Temperature 12°C, pH 6.9 – 7.6. 16 hours light per day. Water hardness and conductivity were 46 mg/L $CaCO_3$ and 200 µmhos/cm, respectively. Dissolved oxygen concentrations were 8.3-8.5 mg/L (77-79% saturation) at test initiation and 3.8-5.6 mg/L (35-52% saturation) at test termination.
Observations:	At 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 were analysed for test substance concentration by HPLC.
Data analysis:	Moving average analysis, probit analysis and nonlinear interpolation.

Results:

Initial difenoconazole concentrations corresponded to 81-107% of nominal concentrations. Mortality data are presented in Table B.9.2.1-1.

Table B.9.2.1-1: Effect of difenoconazole on mortality in rainbow trout

Mean initial neasured concentration (mg/L)	Cumulative mortality (%)			
	24 hour	48 hour	72 hour	96 hour
Control	0	0	0	0
Solvent control	0	0	0	0
0.35	0	0	0	0
0.63	0[b]	0[b]	0[b]	10[b]
1.2	10[a]	20[a]	100	100
1.7	30[a]	100	100	100
2.9	100	100	100	100
4.1	100	100	100	100
LC_{50} (mg/L)	1.8	1.3	0.87	0.81
95% confidence interval (p=0.05)	1.5-2.2	0.63-1.7	0.63-1.2	0.63-1.2

[a] *all surviving fish suffering loss of equilibrium, lethargy and abnormal pigmentation*
[b] *one or more fish suffering loss of equilibrium, lethargy and abnormal pigmentation*

RMS comments:

According to the referred guidelines at least two replicates per treatment level would have been preferred, although not strictly required. The dissolved oxygen concentrations fall below limits of 60% saturation indicated in test guidelines. Only initial test concentrations were analytically verified, and therefore the treatment levels are uncertain. This study will not be further used in the risk assessment.

Reference: **Surprenant, D.C. (1990a). Acute toxicity of CGA 169374 to rainbow trout (*Salmo gairdneri*) under flow-through conditions. [redacted]. [redacted] Unpublished report no. 88-5-2663. (Syngenta File No 169374/0333)**

Test substance:	Technical difenoconazole, batch number FL 851406, purity 96.1%. Prepared in acetone.
Guideline:	US EPA FIFRA 72-1.
GLP:	Yes

Material and methods:

Species:	Rainbow trout (*Salmo gairdneri*), mean weight 0.92 g; mean length 45 mm
Treatments:	Test concentrations 0.45, 0.69, 1.1, 1.6, 2.5 mg/L with a maximum final acetone concentration of 73 µL/L. Two replicate tanks for each concentration, one control tank prepared with 73 µL/L acetone and one control tank.
Number of animals:	Ten fish were introduced to each 15 L tank.
Duration:	96-hours, flow-through (9.2 – 12 tank volume replacements per day)
Test conditions:	Test temperature 11 – 13°C, pH 6.6 – 7.2. 16 hours light per day. The hardness and conductivity of dilution water were 32-33 $CaCO_3$ mg/L and 100-130 µmhos/cm, respectively. Dissolved oxygen concentrations ranged between 7.9 and 11 mg/L over the test duration.
Observations:	At 24, 48, 72 and 96 hours for mortality and abnormal behaviour. 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 HPLC.
Data analysis:	Binominal, moving average and probit tests.

Results:

Mean measured difenoconazole concentrations corresponded to 84-129% of nominal concentrations. Mortality data are presented in Table B.9.2.1-2.

Table B.9.2.1-2: Effect of difenoconazole 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
Solvent control	0	0	0	0
0.58	0[b]	0[b]	0[b]	0[b]
0.78	0[b]	0[b]	0[b]	0[a]
1.1	0[a]	5[b]	30[a]	65[a]
1.4	0[a]	30[a]	70[a]	95[a]
2.1	85[a]	100	100	100
LC_{50} (mg/L)	1.8	1.5	1.2	1.1
95% confidence interval (p=0.05)	1.4-2.1	1.4-1.6	1.1-1.4	0.98-1.1

[a] *all surviving fish suffering loss of equilibrium, lethargy and abnormal pigmentation*
[b] *one or more fish suffering loss of equilibrium, lethargy and abnormal pigmentation*

RMS comment:

The study was conducted in accordance with the referred guidelines and is accepted for the risk assessment. It was noted that no NOEC value could be determined from this study, since sublethal effects were observed at all test concentrations.

Reference:	**Bowman, J.H. (1988). Acute toxicity of CGA 169374 technical to bluegill sunfish (*Lepomis macrochirus*). [redacted] Unpublished report no. 34834. (Syngenta File No 169374/0016)**
Guideline:	US EPA FIFRA 72-1.
GLP:	Yes

Material and methods:

Test substance:	Technical difenoconazole, batch number FL851406, purity 96.1%.
Species:	Bluegill sunfish (*Lepomis macrochirus*)
Treatments:	Aliquots of a difenoconazole stock solution prepared in dimethylformamide, were introduced into tanks to produce 5 exposure concentrations (0.32, 0.56, 1.0, 1.8 and 3.2 mg/L) with a maximum dimethylformamide concentration of 0.11 mL/L. The test incorporated one tank for each exposure concentration, one solvent control tank prepared with 0.11 mL/L dimethylformamide and one control tank.
Number of animals:	Ten fish (mean weight 0.61 g; mean length 29 mm) in each tank.
Duration:	96 hours, static
Test conditions:	Temperature 22 - 23°C, pH 7.0 – 7.5. 16 hours light per day. The hardness and conductivity of dilution water were 40-45 $CaCO_3$ mg/L and 150-170 µmhos/cm, respectively. Dissolved oxygen concentrations were 5.3-8.8 mg/L (60 – 100% of saturation) over the test duration.
Observations:	Mortality and abnormal behaviour was monitored at 24, 48 and 96 hours. Temperature, pH and dissolved oxygen concentrations were also recorded at 24, 48 and 96 hours and water samples collected at test initiation and test termination were analysed for test substance concentration by HPLC.
Data analysis:	Binominal method, moving average method, probit method.

Results:

Initial difenoconazole concentrations corresponded to 109 -118 % of nominal concentrations, and concentrations after 96 hours were 70 – 78% of the nominal values. Mean measured concentrations over the test period were 90 – 97% of nominal concentrations. Mortality data are presented in the table below.

Table B.9.2.1-3: Effect of difenoconazole on mortality in bluegill sunfish.

Nominal concentration (mg/L)	Cumulative mortality (%)			
	24 hour	48 hour	72 hour	96 hour
Control	0	0	0	0
Solvent control	0	0	0	0
0.32	0	0	0	0
0.56	0	0	0	0
1.0	0	0	0 [b]	0 [b]
1.8	0 [a]	0 [a]	70 [a]	100
3.2	60 [a]	100	100	100
LC_{50} (mg/L)	2.7	2.2	n.c.	1.2
95% confidence interval (p=0.05)	n.c.	1.7-2.9	n.c.	0.9-1.7

[a] all surviving fish suffering loss of equilibrium, lethargy and abnormal pigmentation
[b] one or more fish suffering loss of equilibrium, lethargy and abnormal pigmentation
n.c. not calculated.

Based on nominal concentrations, the 96-hour LC_{50} for difenoconazole in bluegill sunfish was reported to be 1.2 mg/L.

RMS comments:

The study was conducted in accordance with the referred guidelines, although more than one replicate would have been preferred. Measured concentrations at termination of the test were low, but since the mean measured were >80%, and therefore calculations based on nominal concentrations are considered to be acceptable. 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 if the concentration interval is less 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}. Hence the LC_{50} from this study can be considered to be 1.3 mg/L.

Reference:	**Ward, G.S. (1988). Acute toxicity of CGA 169374 to the sheepshead minnow (*Cyprinodon variegates*) [redacted] Unpublished report no. 86362-0100-2130. (Syngenta File No 169374/0019)**
Guideline:	US EPA FIFRA 72-3.
GLP:	Yes
Material and methods:	
Test substance:	Technical difenoconazole, batch number FL 851406, purity 96.1%.
Species:	Sheepshead minnow (*Cyprinodon variegates*)
Treatments:	Aliquots of a difenoconazole stock solution prepared in acetone, were introduced into tanks to produce 5 exposure concentrations (0.13, 0.216, 0.36, 0.6 and 1.0 mg/L) with a maximum acetone concentration of 0.5 mL/L. The test incorporated one tank for each exposure concentration, one solvent control tank prepared with 0.5 mL/L acetone and one control tank.
Number of animals:	Ten fish (mean weight 0.003 g; mean length 6.5 mm) were introduced into each tank.
Duration:	96 hours, static test
Test conditions:	Temperature 21 – 22°C, pH 7.7 – 8.2. Salinity 20‰, 14 hours light per day. Dissolved oxygen concentrations were 6.3-7.1 mg/L (ca 70 – 80% of saturation) over the test duration.
Observations:	Mortality and abnormal behaviour was monitored after 24, 48, 72 and 96 hours. Temperature, pH and dissolved oxygen concentrations were also recorded daily and water samples, collected at test initiation, were analysed for test substance concentration by HPLC.
Data analysis:	Binominal method, moving average method, probit method.

Results:

Mean initial difenoconazole concentrations corresponded to 84-150 % of nominal concentrations. Mortality data are presented in the table below.

Table B.9.2.1-4: Effect of difenoconazole on mortality in sheepshead minnow.

Mean initial 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
0.109	0	0	0	0
0.325	0	0	0	0
0.428	0	0	10	10
0.698	0	0	0	0
0.838	0[a]	0[a]	10[a]	60[a]
LC_{50} (mg/L)	>0.838	>0.838	>0.838	0.819
95% confidence interval ($p \leq 0.05$)	n.d.	n.d.	n.d.	n.d.

[a] all surviving fish suffering loss of equilibrium and lethargy
n.d. not determined

Based on the initially measured concentrations, the 96-hour LC_{50} for difenoconazole in sheepshead minnow was estimated to be 0.819 mg/L.

RMS comments:

According to the referred guidelines at least two replicates per treatment level would have been preferred, although not strictly required. Analytical measurements were made only at initiation of the test, and therefore the overall exposure levels are uncertain. The results will not be used in the risk assessment.

Reference:	**Machado, M.W. (1993). CGA 169374 – Acute toxicity to sheepshead minnow (*Cyprinodon variegates*) under flow-through conditions. [redacted] Unpublished report no. 93-5-4795. (Syngenta File No 169374/0949)**
Guideline:	US EPA FIFRA 72-3.
GLP:	Yes.
Material and methods:	
Test substance:	Technical difenoconazole, batch number FL 921937, purity 96%.
Species:	Sheepshead minnow (*Cyprinodon variegates*)
Treatments:	Technical difenoconazole prepared in acetone was introduced into tanks using intermittent-flow proportional diluter apparatus, to produce 5 nominal exposure concentrations (0.32, 0.54, 0.9, 1.5, 2.5 mg/L) with a maximum acetone concentration of 1.79 mL/L. The test incorporated two replicate tanks for each concentration, one solvent control tank prepared with 1.79 mL/L acetone and one control tank.
Number of animals:	Ten fish (mean weight 0.3 g; mean length 28 mm) for each tank.
Duration:	96 hours, flow-through.
Test conditions:	Temperature 22°C, pH 7.6 – 7.9. Salinity 31 – 32‰, 16 hours light per day. Dissolved oxygen concentrations were 6 – 7.1 mg/L (ca 80 –