Fludioxonil

NOTIFICATION OF AN ACTIVE SUBSTANCE UNDER COMMISSION REGULATION (EU) 844/2012

DOCUMENT N4

RELEVANCE OF METABOLITES IN GROUNDWATER

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18/1/17	The metabolite SYN545245 is included in the assessment. All amendments are highlighted in yellow.	CGA173506_11750 11 April 2016 updated 18/1/17

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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1 INTRODUCTION

This document assesses the potential relevance of metabolites of fludioxonil with respect to the current guidance for relevance in groundwater[1]. The assessment of relevance of environmental metabolites for groundwater contamination is discussed according to the scheme laid out in this guidance document. The requirements are specified in italicised text in the following sections.

Within this document the term 'major metabolite' is used to describe those that exceed 10% of the applied ^{14}C (AR), or those metabolites which exceed 5% AR on two consecutive occasions or those which do not reach maximum % AR by the end of the study; metabolites below this threshold are termed 'minor'.

2 FATE AND BEHAVIOUR IN THE ENVIRONMENT

2.1 Summary of Degradation pathway in Soil

All metabolites which are expected to occur in soil under normal use conditions, on the basis of results from soil degradation studies and soil lysimeter studies, should be assessed for their ability to contaminate groundwater.

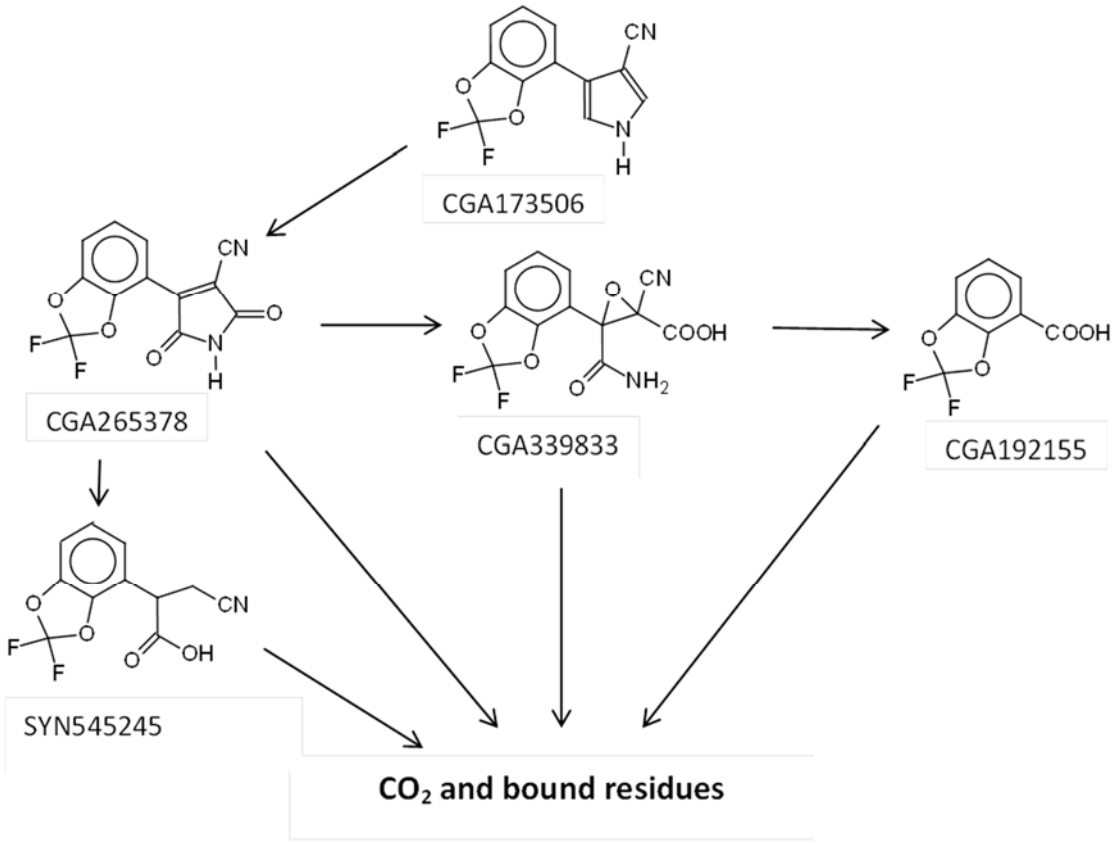
Studies on the degradation of fludioxonil in aerobic soil have been submitted previously for EU review. Additional data have been generated and are presented in **MCA Section 7 Supplement** assessing the Fate & Behaviour in the Environment. The findings from the laboratory aerobic degradation studies indicates that soil degradation in the dark is mainly mediated by biotic processes but that the soil degradation of fludioxonil is slow with no major metabolites being formed in the soil.

Studies on the photolytic degradation of fludioxonil in soil have been submitted previously for EU review. Additional data have been generated and are presented in **MCA Section 7** assessing the Fate & Behaviour in the Environment. The findings from the laboratory photolysis studies indicates that photolytic degradation plays a major role in the transformation of fludioxonil in soil with it being the only soil process that forms major soil metabolites. Photodegradation initially involves oxidation on the carbon 2 and 5 in the pyrrole-ring, leading to CGA265378 (dioxo pyrrole). Subsequently opening of the pyrrole-ring leads to CGA339833 (epoxide) which is then further degraded to CGA192155 (carbocyclic acid). Additionally an alternative photolytic pathway for fludioxonil is the deamination and decarboxylation of the pyrrole-ring in CGA265378 to form SYN545245 (propionic acid).

As no major metabolites have been identified in the dark, the proposed photodegradation pathway for fludioxonil in soil considering the major identified metabolites is shown in Figure 2.1-1.

[1] Sanco/221/2000-rev.10 (25 February 2003) Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated Under Council Directive 91/414/EEC.

Figure 2.1-1: Proposed degradation pathway for fludioxonil in light exposed soil



2.2 Summary of Identification of Metabolites in Soil

The criteria for the identification of metabolites which require further consideration are given in the guidance document (SANCO/221/2000):

SANCO/221/2000: - Degradation products must be characterised and identified by the notifiers to the extent that it is technically feasible and their relevance must be assessed, if one of the following conditions applies:

a) Metabolites, which account for more than 10 % of the amount of active substance added in soil at any time during the studies; or

b) Which account for more than 5 % of the amount of active substance added in soil in at least two sequential measurements during the studies; or

c) For which at the end of soil degradation studies the maximum of formation is not yet reached.

Moreover, all metabolites found in lysimeter studies at annual average concentrations exceeding 0.1 µg/L in the leachate should be identified and subject to further assessment.

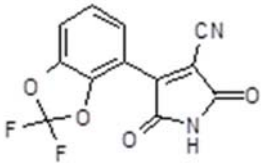
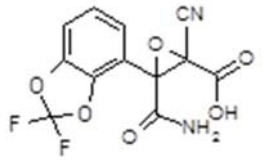
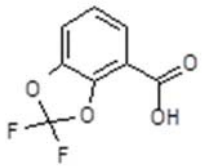
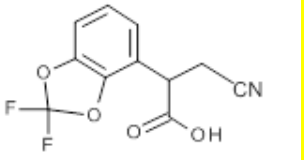
The following metabolites were identified which account for more than 10 % AR during the studies, or metabolites which were more than 5% AR on two consecutive occasions or those which did not reach maximum % AR by the end of the study - see **MCA Section 7 Supplement** assessing the Fate & Behaviour in the Environment.

Table 2.2-1: Maximum concentration values (%) for the main metabolites of fludioxonil

Code of Compound	Maximum occurrence in laboratory photolysis studies [%]
CGA265378	10.1
CGA339833	13.4
CGA192155	18.1
SYN545245	29.4

Therefore the metabolites CGA265378, CGA339833, SYN545245 and CGA192155 are assessed in this document for potential relevance to groundwater. ~~Aerobic soil rate and adsorption/desorption data is currently being generated for SYN545245 and this document will be updated when that data are available~~. For information details of the occurrence of these assessed metabolites in soils or water as well as their occurrence in crops, rats, goats and/or hens are given in Table 2.2-2 below.

Table 2.2-2: Occurrence of potentially relevant metabolites of fludioxonil in soils, water or crops as well as their occurrence in rats, goats and/or hens

Metabolite	Structure	Soil	Water	Crops	Rat	Goat	Hen
CGA265378		X		X(a,b)			X
CGA339833		X	X	X(a,b)			
CGA192155		X	X	X(a,b)			X
SYN545245		X					

(a) Primary crops
(b) Rotational crops

3 RELEVANCE OF METABOLITES IN GROUNDWATER

3.1 Step 1: Exclusion of Degradation Products of No Concern

General criteria for exclusion of potentially relevant metabolites for consideration are given in the guidance document:

SANCO/221/2000: - A degradation product which may be expected to occur in groundwater as a result of a soil degradation study or a lysimeter study will require further assessment unless one of the following conditions apply:

a) it is CO_2 or an inorganic compound, not containing a heavy metal; or,

b) it is an organic compound of aliphatic structure, with a chain length of 4 or less, which consists only of C, H, N or O atoms and which has no "alerting structures" such as epoxide, nitrosamine, nitrile or other functional groups of known toxicological concern.

c) it is a substance, which is known to be of no toxicological or ecotoxicological concern and which is naturally occurring at much higher concentrations in the respective compartment.

The metabolites CGA265378, CGA339833, SYN545245 and CGA192155 were identified in laboratory soil photolysis studies and do not meet the above conditions and therefore will each require further assessment.

3.2 Step 2: Quantification of Potential Groundwater Contamination

Step 2 of the guidance document (SANCO/221/2000) requires consideration of FOCUS groundwater models and scenarios and results of lysimeter studies:

The models PEARL v 4.4.4, PELMO v 5.5.3 and MACRO v 5.5.4 and the FOCUS groundwater scenarios were used to predict the leaching behaviour of fludioxonil and its soil photolysis metabolites following a foliar application (the photolytic metabolites are not relevant for seed treatment applications). The results of modelling in the environmental compartments are given in the appropriate **MCP Section 9 (A8240D)** assessing the Fate & Behaviour in the Environment.

The PEC of fludioxonil, CGA265378, CGA339833, SYN545245 and CGA192155 in groundwater (PEC_{GW}) has been assessed with a representative use pattern of 2 x 500 g/ha in vines, 2 x 225 g/ha in apples and 2 x 250 g/ha in strawberries.

Maximum predicted PEC_{GW} from FOCUS PEARL, PELMO and MACRO following application of fludioxonil to vines, apples and strawberries are shown in Tables 3.2-1 to Table 3.2-8. For MACRO, only the scenario Châteaudun is defined for vine and apple. Strawberry is not parameterised in Châteaudun, therefore no simulations using FOCUS-MACRO for strawberries were performed in accordance with FOCUS guidance.

Vines

Table 3.2-1: Overall maximum PEC_{GW} of fludioxonil, CGA265378, CGA339833, SYN545245 and CGA192155 for the use patterns in vines (FOCUS-PEARL)

Crop	Application rate [g a.s./ha]	BBCH at first application [approx.]	PEC_{GW} at 1 m soil depth [µg/L]				
			Fludioxonil	CGA265378	CGA339833	CGA192155	SYN545245
Vines	2 x 500 g a.s./ha	67	< 0.001	<0.001	3.50	0.127	<0.001

Table 3.2-2: Overall maximum PEC_{GW} of fludioxonil, CGA265378, CGA339833, SYN545245 and CGA192155 for the use patterns in vines (FOCUS-PELMO)

Crop	Application rate [g a.s./ha]	BBCH at first application [approx.]	PEC_{GW} at 1 m soil depth [µg/L]				
			Fludioxonil	CGA265378	CGA339833	CGA192155	SYN545245
Vines	2 x 500 g a.s./ha	67	< 0.001	<0.001	3.74	0.201	<0.001

Table 3.2-3: Overall maximum PEC_{GW} of fludioxonil, CGA265378, CGA339833, SYN545245 and CGA192155 for the use patterns in vines (FOCUS-MACRO)

Crop	Application rate [g a.s./ha]	BBCH at first application [approx.]	PEC_{GW} at 1 m soil depth [µg/L]				
			Fludioxonil	CGA265378	CGA339833	CGA192155	SYN545245
Vines	2 x 500 g a.s./ha	67	< 0.001	<0.001	0.912	0.010	<0.001

Apples

Table 3.2-4: Overall maximum PEC_{GW} of fludioxonil, CGA265378, CGA339833, SYN545245 and CGA192155 for the use patterns in apples (FOCUS-PEARL)

Crop	Application rate [g a.s./ha]	BBCH at first application [approx.]	PEC_{GW} at 1 m soil depth [µg/L]				
			Fludioxonil	CGA265378	CGA339833	CGA192155	SYN545245
Apples	2 x 225 g a.s./ha	74	< 0.001	<0.001	2.54	0.033	<0.001

Table 3.2-5: Overall maximum PEC_{GW} of fludioxonil, CGA265378, CGA339833, SYN545245 and CGA192155 for the use patterns in apples (FOCUS-PELMO)

Crop	Application rate [g a.s./ha]	BBCH at first application [approx.]	PEC_{GW} at 1 m soil depth [µg/L]				
			Fludioxonil	CGA265378	CGA339833	CGA192155	SYN545245
Apples	2 x 225 g a.s./ha	74	< 0.001	<0.001	2.51	0.055	<0.001

Table 3.2-6: Overall maximum PEC_{GW} of fludioxonil, CGA265378, CGA339833, SYN545245 and CGA192155 for the use patterns in apples (FOCUS-MACRO)

Crop	Application rate [g a.s./ha]	BBCH at first application [approx.]	PEC_{GW} at 1 m soil depth [µg/L]				
			Fludioxonil	CGA265378	CGA339833	CGA192155	SYN545245
Apples	2 x 225 g a.s./ha	74	< 0.001	<0.001	0.148	< 0.001	<0.001

Strawberries

Table 3.2-7: Overall maximum PEC_{GW} of fludioxonil, CGA265378, CGA339833, SYN545245 and CGA192155 for the use patterns in strawberries (FOCUS-PEARL)

Crop	Application rate [g a.s./ha]	BBCH at first application [approx.]	PEC_{GW} at 1 m soil depth [µg/L]				
			Fludioxonil	CGA265378	CGA339833	CGA192155	SYN545245
Strawberries	2 x 250 g a.s./ha	61	< 0.001	<0.001	0.906	0.007	<0.001

Table 3.2-8: Overall maximum PEC_{GW} of fludioxonil, CGA265378, CGA339833, SYN545245 and CGA192155 for the use patterns in strawberries (FOCUS-PELMO)

Crop	Application rate [g a.s./ha]	BBCH at first application [approx.]	PEC_{GW} at 1 m soil depth [µg/L]				
			Fludioxonil	CGA265378	CGA339833	CGA192155	SYN545245
Strawberries	2 x 250 g a.s./ha	61	< 0.001	<0.001	0.967	0.007	<0.001

The predicted environmental concentrations (PEC_{GW}) at 1m depth for fludioxonil and metabolites CGA265378 and SYN545245 following 20 years use on vines, apples and strawberries at representative use patterns of 2 x 500 g/ha, 2 x 225 g/ha and 2 x 225 g/ha, respectively, were less than 0.1 µg/L in all scenarios using FOCUS-PELMO, PEARL and MACRO models.

The models predict that metabolites CGA339833 (vines, apples and strawberries) and CGA192155 (vines) will be found in groundwater at concentrations greater than 0.1 µg/L.

The predicted concentrations of CGA265378 and SYN545245 were below the currently specified limit for groundwater intended for the production of drinking water laid down in Directive 80/778/EEC, in each of the FOCUS scenarios. Therefore, further assessment of the potential relevance of ~~this compound is~~ these compounds are not required.

The predicted concentrations of CGA339833 and CGA192155 were above the currently specified limit for groundwater intended for the production of drinking water laid down in Directive 80/778/EEC, for some of the FOCUS scenarios. Therefore, further assessment of the potential relevance of these compounds is required.

3.3 Step 3: Hazard Assessment: Identification of relevant metabolites

Step 3 of the guidance document (SANCO/221/2000) involves assessment of biological activity, genotoxicity and toxicity of a major metabolite which may potentially exceed 0.1 µg/L in groundwater.

3.3.1 Step 3, Stage 1: Screening for biological activity

SANCO/221/2000: - metabolites with a comparable or higher biological activity than the parent are considered as relevant and must, therefore, not exceed a level of 0.1 µg/L in groundwater …. In screening assays …it should be sufficient to demonstrate that the biological activity of a metabolite is clearly less that 50% of the activity of the parent molecule. Otherwise the biological activity should be considered as "comparable".

According to the tiered approach in SANCO/221/2000 - Rev 10, the fungicidal activity of metabolites identified in Step 2 as having the potential to exceed 0.1 µg/L in groundwater (see Tables 3.2-1 to 3.2-8) was measured relative to the parent compound against representative fungal pathogen species. Results are summarised below (Table 3.3.1-1). Full study summaries are available in the **MCA Section 8 Supplement**.

Table 3.3.1-1: Summary of biological activity of fludioxonil metabolites

Metabolite code	Use rate (g a.s./ha)	Fungal species tested (pathosystem)	Activity relative to parent (%)	Test result/ Conclusion	Reference (author, date, Syngenta Ref)
CGA192155	200 g/L	*Erysiphe graminis* (Barley)	50 [b]	Weak activity [a]	***Zeun (2002)*** [c] ***CGA173506/5548 (study not considered fully acceptable in DAR)***
		Botrytis cinerea (Apple)	0	Not biologically active	
		Pyriculari culmorum (Rice)	0		
	150	*Botrytis cinerea* (Oilseed rape)	0	Not biologically active	***Haas (2015) CGA173506/11931***
	60	*Alternaria solani* (Tomato)	10		***Schneiter (2015) CGA173506/11933***
CGA339833	200 g/L	*Erysiphe graminis* (Barley)	0	Not biologically active	***Zeun (2002) CGA173506/5548 (study not considered fully acceptable in DAR)***
		Botrytis cinerea (Apple)	0		
		Pyriculari culmorum (Rice)	0		
	20 g/L	*Fusarium culmoram* (Wheat)	0		
	150	*Botrytis cinerea* (Oilseed rape)	0	Not biologically active	***Haas (2015) CGA173506/11931***
	60	*Alternaria solani* (Tomato)	0		***Schneiter (2015) CGA173506/11933***

[a] Classified as having weak activity to *Erysiphe graminis* with no activity to the other 2 species of fungi; the rate tested was very high.
[b] Originally recorded on a 0 to 10 scale, expressed as percentage (%) for consistency with other data shown in table.
[c] Disease levels in these tests conducted at that time were on average are >80% (Pers. Comm with R. Zeun).

Syngenta has also generated biological activity data for CGA265378 which shows no activity relative to parent. These data are included in the references cited above but have not been summarised as CGA265378 does not trigger step 3 assessment.

3.3.2 Step 3, Stage 2: Screening for genotoxicity

SANCO/221/2000: - All metabolites should be screened for their genotoxic activity by at least the following package of in vitro genotoxicity studies: Ames test, gene mutation test with mammalian cells and chromosome aberration test. Equivocal results in in vitro studies should be substantiated by in vivo experiments.

Metabolites CGA192155 and CGA339833 required further evaluation. The genotoxicity data are presented below. In addition, the structures were considered for Quantitative Structure Activity Relationship (QSAR) using the predictive toxicology program DEREK. DEREK, an acronym for **D**eductive **E**stimation of **R**isk from **E**xisting **K**nowledge, is a database that searches against a number of predictive endpoints, specifically thyroid toxicity, carcinogenicity, mutagenicity, irritation, respiratory sensitisation and skin sensitisation.

Genotoxicity data on CGA192155:

CGA192155 was not mutagenic in the gene mutation assays (Ames test and mouse lymphoma cell L5178Y assay). It gave a positive result in the chromosome aberration test; but in the confirmatory *in vivo* micronucleus test the outcome was negative, see Table 3.3.2-1 below.

Table 3.3.2-1: Summary of Genotoxicity data on CGA192155

CGA192155			
Study	Test Object	Concentration	Results
Ames test **[Hertner, 1993, (Report no. 933001)]**	*S.typhimurium* and *E.coli*	312.5-5000 µg/plate	negative
Chromosome aberration test **[Bohnenberger, 2007, (Report no. 1076601)]**	Human lymphocytes	4-22h: 378.8-1160 µg/ml (-S9), 4-22h: 378.8-1160 µg/ml (+S9)	positive
Gene mutation in mammalian cells **[Sokolowski, 2007, (Report No. 107662)]**	Mouse lymphoma cells L5178Y	4h: 63.1-2020 µg/ml (-S9), 63.1-2020 µg/ml (+S9)	negative
In vivo micronucleus test **[Honarvar, 2007, (Report no. 1121700)]**	Mouse bone marrow	500, 1000 and 2000 mg/kg bw	negative

QSAR (DEREK) indicates no structural alerts.

Genotoxicity data on CGA339833:

CGA339833 was not mutagenic in bacterial Salmonella and Escherichia gene mutation assays.

In the mouse lymphoma cell L5178Y gene mutation assay CGA339833 gave a borderline increase in mutant frequency at a single concentration in the absence of metabolic activation only. This slight increase occurred in only one of two parallel experiments and was associated with a high level of cellular toxicity at the 24 hour timepoint only; no increases were observed in the presence or absence of metabolic activation after 4 hours. The effect seen at 24 hours occurred at a concentration (2400 µg/mL) above that currently specified by the OECD test guideline (OECD 490 2015); [CGA339833 (MW 312)] requiring testing to a maximum concentration of 10 mM or 2000 µg/mL, hence the maximum required concentration would currently be 2000 µg/mL], where a low relative total growth is evident (13.4%) and at a timepoint (24 h) that is not routinely required in this assay. The combination of an effect only seen in the presence of high cytotoxicity at a concentration in excess of current testing requirements brings its relevance into question. Syngenta's opinion is that due to the lack of effects at <2400 µg/mL Fludioxonil is not mutagenic in this assay at the relevant concentration and timepoint.

A negative result is supported by the test compound's chemical properties which showed an exceptionally low nucleophilic reactivity of CGA339833's epoxide functionally and therefore a likely lack of reactivity towards DNA. CGA339833 showed clastogenic potential in an *in vitro* chromosomal aberration test in Chinese hamster V79 cells but at concentrations which were also cytotoxic to cells. The observed effects are considered attributable to cytotoxicity rather than a direct action on DNA.

Table 3.3.2-2: Summary of Genotoxicity data on CGA339833

CGA339833			
Study	Test Object	Concentration	Results
Ames test **[Deparade, 2000, (Report no. 20003047)]**	*S.typhimurium* and *E.coli*	312.5-5000 µg/plate	negative
Gene mutation in mammalian cells **[Wollny, 2001, (Report No. 680501)]**	Mouse lymphoma cells L5178Y	4h: 400-3200 µg/ml (-S9), 200-3400 µg/ml (+S9) 24h: 400-3200 µg/ml (-S9)	negative
In vitro cytogenetic test **[Schulz 2002 (Report no. 20003058)]**	Chinese hamster V79 cells	-S9: 800-2400 µg/ml (4h+14h), 200-1600 µg/ml (18h), 800 µg/ml (28h); +S9: 200-2400 µg/ml (4h+14h), 200-800 µg/ml (4h+24h)	positive: -S9: 18h negative: -S9: 4h (+14h) and 28h +S9: 4h (+14h) and 4h (+24h)
In vivo micronucleus test **[Fox 2002 (Report no. SR1164)]**	Rat bone marrow	500, 1000 and 2000 mg/kg bw	negative

This view the mutagenic and clastogenic effects are considered attributable to cytotoxicity is supported by the evidence of CGA339833's high stability and resistance to nucleophilic attack and therefore likely lack of DNA reactivity. This position is summarised in ***[Watson,W, 2005, Document ERA 12727, Syngenta File No. CGA173506_11650].*** CGA339833 did not show clastogenic or aneugenic potential in an *in vivo* micronucleus test in rat bone marrow. On the basis of the genotoxicity tests performed coupled with CGA339833's physicochemical properties the compound is considered non genotoxic.

QSAR (DEREK) revealed structural alerts for mutagenicity and carcinogenicity due to the presence of the epoxide group. The absence of a relevant mutagenic potential in the four genotoxicity studies reveals that the DEREK alert for mutagenicity/carcinogenicity is a false positive.

Genotoxicity data on CGA265378:

Ames test and acute oral toxicity studies for CGA265378 were evaluated in the original EU review (Hartmann 1992 and Hertner 1992). Syngenta has also generated additional genotoxicity data for CGA265378 which shows that this metabolite is non-genotoxic. These data have not been submitted since CGA265378 does not trigger step 3 assessment. For completeness, the studies are included in the unsubmitted list in the **LCA Section 5 Supplement**.

3.4 Step 3, Stage 3: Screening for toxicity

SANCO/221/2000: - A metabolite is considered "relevant" if its toxicological properties lead to a classification as toxic or very toxic (T or T+) according to Directive 67/548/EEC. ……the toxicity classification of the parent active substance as determined according to Directive 67/548/EEC is used for pragmatic reasons as a starting point to focus the screening activity…… All metabolites passing stage 3 of step 3 and are not considered as "relevant" are subject to an exposure and/or risk assessment….

Fludioxonil has no classification for health hazards according to according to Regulation (EC) No 1272/2008 as amended (**MCA Section 10 Supplement**)

Toxicity data on CGA192155:

The toxicity data for metabolite CGA192155 are summarised in Table 3.3.3-1 below:

Table 3.3.3-1: Toxicity data on CGA192155

CGA192155:			
Study	Species	Dose level	Result
Acute oral toxicity ***[Hartmann 1993 (Report No. 933000]***	rat	2000 mg/kg bw	LD50 > 2000 mg/kg bw NOEL 2000 mg/kg bw
28 day feeding ***[Harder 2008 (Report No. 18966)]***	rat	0, 1000, 5000 or 15000 ppm equivalent to 78, 382 and 1147 mg/kg bw/day for males and 80, 389 and 1065 mg/kg bw/day for females	NOAEL 1000 ppm ~ 78/80 mg/kg bw/day Findings 15000ppm: reduced bw development and food consumption; liver: increased weight. Changes in haematology and clinical chemistry parameters but no histopathological findings 5000ppm: slight haematological changes.
90day feeding ***[Harder 2008 (Report No. 18977)]***	rat	0, 100, 1000 or 7000 ppm equivalent to 5.9, 57.5 and 414.7 mg/kg bw/day for males and 6.7, 66.2 and 461.2 mg/kg bw/day for females	NOAEL 1000 ppm ~ 57.5/66.2 mg/kg bw/day Findings 7000ppm: reduced bw development and food consumption. No changes in haematology and clinical chemistry parameters nor histopathological finding.

Toxicity data on CGA339833:

The toxicity data for metabolite CGA339833 are summarised in Table 3.3.3-2 below:

Table 3.3.3-2: Toxicity data on CGA339833

CGA339833:			
Study	Species	Dose level	Result
Acute oral toxicity ***[Sommer 2000 (Report No. 20003046)]***	rat	2000 mg/kg bw	LD50 > 2000 mg/kg bw NOEL 2000 mg/kg bw
90 day feeding ***[Sommer 2001 (Report No. 20003048)]***	rat	0, 10, 100, 800, 2500 and 7000 ppm equivalent to 0.7, 7.1, 58, 185 and 513 mg/kg bw/day for males and 0.9, 8.7, 66.6, 208 and 601 mg/kg bw/day for females	NOEL(m) 100 ppm ~ 7.1 mg/kg bw/day NOAEL(m+f) = NOEL(f) 800 ppm ~ 58 / 66.6 mg/kg bw/day for males / females Findings LOAEL: reduced bw development; liver: increased weight, hepatocellular hypertrophy, kidney: increased weight, tubular casts (males); olfactory epithelium atrophy

QSAR (DEREK) revealed structural alerts for skin sensitisation and irritation of skin and eye due to the presence of the epoxide group. These are not relevant endpoints for water or soil metabolites, as human exposure to relevant amounts of the compound via skin or eyes will not occur. Furthermore, the DEREK alert for sensitisation/irritation may also be false positive, as the epoxide group they are based on has been shown (by the genotoxicity studies) not to exhibit the activity that would have been expected for an unsubstituted epoxide.

Comment on comparative toxicity of metabolites CGA192155 and CGA339833 versus parent fludioxonil:

Comparison of the toxicity data for CGA192155 and CGA339833 and parent fludioxonil shows that the metabolites are no more toxic than the parent.

Table 3.3.3-3: Comparison of Toxicity Profile for CGA192155 and CGA339833 with Fludioxonil

Study	Fludioxonil	CGA192155	CGA339833
Acute Oral	>5000 mg/kg	>2000 mg/kg	>2000 mg/kg
90 Day Sub-chronic (oral)	NOAEL = 64 mg/kg bw/day	NOAEL = 57.5 mg/kg bw/day	NOAEL = 58 mg/kg bw/day
Point Mutation Assay	Negative	Negative	Negative
Chromosome Aberation Assay	Positive *	Positive *	Negative (+S9) Positive (-S9)
Mammalian Cell Gene Mutation	Negative	Negative	Negative
In vivo rodent micronucleus assay	Negative#	Negative	Negative

1 of the 5 in vivo chromosome aberration tests performed was considered equivocal. The relevance of this study is described briefly in section MCA Section 5 Supplement, and it has been superceded by a more recent (and robust) study. Overall, it is considered that fludioxonil yields a negative outcome in the genotoxicity assays.

Like parent fludioxonil, the metabolites are of low acute oral toxicity. Similar effects and NOAELs were seen in rats following sub-chronic administration of CGA339833 for up to 90 days as those that were observed for fludioxonil i.e. reduced body weight gain, effects on the liver (increased weight, hepatocyte hypertrophy) and the kidney (tubular casts predominantly in males). Unlike fludioxonil, minimal to slight atrophy of the olfactory epithelium at the two top dose levels of CGA339833 was seen (2500 and 7000 ppm), however the observed effects were high dose effects of a low severity with a clear NOEL. CGA192155 only exhibited decreased bodyweight and food consumption at the highest doses in the 90 day study. In summary, CGA192155 or CGA339833 are no more toxic than parent fludioxonil and therefore not toxicologically relevant.

3.5 Step 4: Exposure assessment – threshold of concern approach

Step 4 of the guidance document (SANCO/221/2000) involves an exposure assessment:

SANCO/221/2000: - Metabolites which have not been identified as being relevant according to the screening outlined in step 3, should be further tested in an exposure assessment to make sure that any contamination of groundwater will not lead to unacceptable exposure of consumers via their drinking water.

For metabolites appearing in groundwater at a concentration below 0.75 µg/L, which pass the screening for biological activity, genotoxicity and toxicity, the SANCO guidance document foresees a threshold of concern approach. The threshold has been set at 0.75 µg/L based on a large pool of toxicological reference data. Following this approach, a toxicological threshold of 1.5 µg/person/day can be derived for individual degradates, including those of unknown structure. This value corresponds to an upper limit

of 0.75 µg/L of exposure for humans for each metabolite assuming the consumption of 2 litres of drinking water per day which contains residues of the metabolite at the threshold level.

CGA192155 does not exceed the 0.75 µg/L limit in the modelling scenarios presented for the representative uses. Therefore, a refined exposure and risk assessment according to Step 5 is not required. However, a refined risk assessment is proposed so that an ADI can be defined in the list of endpoints since a refined risk assessment may be necessary to support additional uses of fludioxonil.

CGA339833 does exceed 0.75 µg/L in some modelled scenarios. Therefore, a refined exposure and risk assessment according to Step 5 is required.

3.6 Step 5: Refined risk assessment for non-relevance of metabolites

Step 5 of the guidance document (SANCO/221/2000) involves a refined risk assessment:

SANCO/221/2000: - Metabolites which have passed steps 1 to 3 and for which levels of estimated concentrations of metabolites in groundwater (as defined in Step 2) lie between 0.75 µg/L (from Step 4) and 10 µg/L will require a refined assessment of their potential toxicological significance for consumers.

CGA339833

CGA339833 has a PEC_{gw} between 0.75 µg/L and 10 µg/L in some scenarios. A refined assessment of the potential toxicological significance including the selected ADI is presented here.

Extensive toxicological testing was conducted on CGA339833 which allows the derivation of an appropriate ADI (acceptable daily intake) to carry out a consumer risk assessment. Since CGA339833 is considered to have no genotoxic potential this value can be derived from the 90-day subchronic test by considering a safety factor of 1000 in the absence of chronic studies.

Table 3.5-1: Refined risk assessment – Derivation of acceptable daily intake (ADI)

Metabolite	Toxicity studies	SF	ADI
CGA339833	90 day sub-chronic (oral) NOAEL = 58 mg/kg bw/day	1000*	0.058 mg/kg bw/day

* - 100 fold inter & intraspecies safety factor & additional 10 fold safety factor for extrapolation to chronic exposure

For the refined risk assessment the ADI value is compared with the theoretical maximum daily intake (TMDI) for CGA339833 which is derived from the maximum potential exposure via groundwater. The highest concentration of CGA339833 estimated in the FOCUS groundwater modelling was at 3.74 µg/L. The TMDI is based on the maximum residues in groundwater and the assumption that the daily water consumption of a 60 kg individual is 2 litres.

The maximum potential exposure of CGA339833 via ground water would be 0.21 % of the ADI.

Calculation of risk (% ADI) for 60-kg adult (consuming 2.0 L/day):

Maximum residue in ground water (µg/L)	Water consumption (L/day)	Individual body weight (kg)	TMDI (mg/kg bw/day)	% of ADI
3.74	2	60	0.0001247	0.21 %

In conclusion, levels of exposure of CGA339833 which has the potential to exceed 0.75 µg/L in groundwater at 1m depth, are far below the established ADI and do not present a risk to human health.

It is concluded that CGA339833 is therefore a non-relevant metabolite with regard to potential for ground water contamination.

CGA192155

CGA192155 has a PEC_{gw} between 0.1 µg/L and 0.75 µg/L in some scenarios. Although a refined risk assessment for CGA192155 is not triggered for the uses presented in this supplementary dossier, it is anticipated that this may be required for some additional uses of fludioxonil and therefore a refined risk assessment of the potential toxicological significance including the selected ADI is presented here.

Extensive toxicological testing was conducted on CGA192155 which allows the derivation of an appropriate ADI (acceptable daily intake) to carry out a consumer risk assessment. Since CGA192155 is considered to have no genotoxic potential this value can be derived from the 90-day subchronic test by considering a safety factor of 1000 in the absence of chronic studies.

Table 3.5-1: Refined risk assessment – Derivation of acceptable daily intake (ADI)

Metabolite	Toxicity studies	SF	ADI
CGA192155	90day subchronic (oral) NOAEL 57.5 mg/kg bw/day	1000*	0.0575 mg/kg bw/day

* - 100 fold inter & intraspecies safety factor & additional 10 fold safety factor for extrapolation to chronic exposure

For the refined risk assessment the ADI value is compared with the theoretical maximum daily intake (TMDI) for CGA192155 which is derived from the maximum potential exposure via groundwater. The highest concentration of CGA192155 estimated in the FOCUS groundwater modelling was at 0.201 µg/L. The TMDI is based on the maximum residues in groundwater and the assumption that the daily water consumption of a 60 kg individual is 2 litres.

The maximum potential exposure of CGA192155 via ground water would be 0.01 % of the ADI.

Calculation of risk (% ADI) for 60-kg adult (consuming 2.0 L/day):

Maximum residue in ground water (µg/L)	Water consumption (L/day)	Individual body weight (kg)	TMDI (mg/kg bw/day)	% of ADI
0.201	2	60	0.000007	0.01 %

In conclusion, predicted levels of exposure of CGA192155 in groundwater at 1m depth, are far below the established ADI and do not present a risk to human health.

It is concluded that CGA192155 is therefore a non-relevant metabolite with regard to potential for ground water contamination.

3.7 Overall Conclusion

Consequently all the metabolites discussed can be considered to be <u>non-relevant</u> in the context of the criteria outlined in the "Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated Under Council Directive 91/414/EEC. (SANCO/221/2000-rev.10; 25 February 2003).

4 REFERENCES

All data are provided in the relevant section/data point of the K document and the report summary in the associated M-CA document summary.