REGISTRATION REPORT

Part B

Section 10

Assessment of the relevance of metabolites in groundwater

Detailed summary of the risk assessment

Product code: BAS 736 00 F

Product name(s): **Miralon**

Chemical active substance(s):

Fluxapyroxad, 50 g/L
Azoxystrobin, 75 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(new authorization)

Applicant: BASF

Submission date: 12/2021

Evaluation date: September 2022

MS Finalisation date: dd/mm/yyyy

Version history

When	What
12/2021	Initial dRR - BASF DocID 2020/2101180
09/2022	Version evaluated by zRMS PL

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10 Relevance of metabolites in groundwater

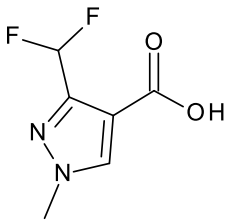
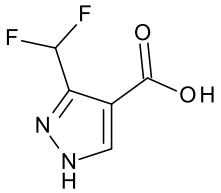
10.1 General information

Fluxapyroxad

The metabolites M700F001 and M700F002 of fluxapyroxad are predicted to occur in groundwater at concentrations above 0.1 µg L^{-1} (please see **BAS 736 00 F, Part B, Central core, Section 8.8.2** and Table 10.1-1 below). Assessment of the relevance of these metabolites according to the stepwise procedure of the EC guidance document SANCO/221/2000 –rev.10 is therefore required.

General information on the metabolites is provided in Table 10.1-1. The impact of the relevance assessment on whether a particular GAP use leads to acceptable risk or not is presented in the summary of the critical GAP evaluation in Chapter 8.1 of the dRR Part B, Section 8 (Environmental fate and behaviour).

Table 10.1-1: General information on the metabolite(s)

Name of active substance	Metabolite name and code	Structural/molecular formula	Trigger for relevance assessment	
Fluxapyroxad BAS 700 F	M700F001		Max PEC_{gw} Based on:	0.292 µg L^{-1} Crop: spring cereals, $FOCUS_{gw}$ scenario: Jokioinen, model: FOCUS-PELMO 5.5.3, slow phase degradation
Fluxapyroxad BAS 700 F	M700F002		Max PEC_{gw} Based on:	2.838 µg L^{-1} Crop: winter cereals, $FOCUS_{gw}$ scenario: Jokioinen, model: FOCUS-PEARL 4.4.4, fast phase degradation

Review Comments:
Based on the results of FOCUS groundwater PEC_{gw} calculated for fluxapyroxad do not exceed the regulatory trigger of 0.1 µg/L at 1 m depth in any of the scenarios.
However, PEC_{gw} for both fluxapyroxad metabolites exceed this threshold. The maximum PEC_{GW} of M700F001 and M700F002 were 0.292 µg/L and 2.838 µg/L, respectively.

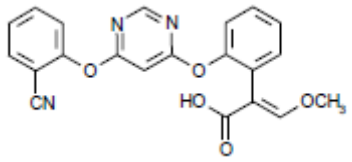
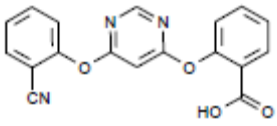
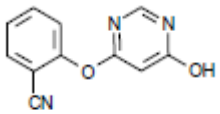
Azoxystrobin

The PEC_{GW} of the azoxystrobin metabolites R402173 and R401553 were < 0.1 µg/L in the relevant application patterns (please see **BAS 736 00 F, Part B, Central core, Section 8.8.2** and Table 10.1-2 below). No assessment is thus required for these metabolites.

The azoxystrobin metabolite R234886 is predicted to occur in groundwater at concentrations above 0.1 µg/L (please see **BAS 736 00 F, Part B, Part B, Central core, Section 8.8.2** and Table 10.1-2 below). Assessment of the relevance of this metabolite according to the stepwise procedure of the EC guidance document SANCO/221/2000 –rev.10 is therefore required.

General information on the metabolites are provided in Table 10.1-2. The impact of the relevance assessment on whether a particular GAP use leads to acceptable risk or not is presented in the summary of the cGAP evaluation in Part B, Section 8 (Environmental fate and behaviour).

Table 10.1-2: General information on the metabolites

Name of active substance	Metabolite name and code	Structural/molecular formula	Trigger for relevance assessment [a]	
Azoxystrobin	R234886		Max PEC_{GW} Based on:	0.513 µg/L Crop: spring cereals, $FOCUS_{gw}$ scenario: Hamburg, model: FOCUS-PEARL 4.4.4, Tier 2 alkaline
	R402173		Max PEC_{GW} Based on:	<0.001 µg/L Crop: spring and winter cereals, $FOCUS_{gw}$ scenarios: all, model: FOCUS-PEARL (v4.4.4), FOCUS-PELMO (v5.5.3), FOCUS-MACRO (v5.5.4)
	R401553		Max PEC_{GW} Based on:	0.001 µg/L Crop: spring cereals, $FOCUS_{gw}$ scenarios: Okehampton, model: FOCUS-PELMO (v5.5.3)

Review Comments:
Based on the results of FOCUS groundwater PEC_{gw} calculated for azoxystrobin do not exceed the regulatory trigger of 0.1 µg/L at 1 m depth in any of the scenarios.
The maximum PEC_{GW} of R401553 and R402173 were below 0.1 µg/L in all scenarios. As the sorption of metabolites R234886 is pH dependent, the lowest K_{foc} and associated 1/n values from the sorption datasets for metabolite were selected for input as a worst-case at Tier 1. Further simulations were performed for metabolite R234886 at Tier 2 using scenario specific K_{foc} values, which were derived using regression analysis. The maximum Tier 2 PEC_{GW} was 0.513 µg/L.

10.2 Relevance assessment of M700F001

The relevance of the groundwater metabolite M700F001 has already been assessed and the assessment agreed at EU level (see EFSA conclusion on fluxapyroxad, 2012), and the relevance assessment is applicable as well for the GAP and groundwater scenarios considered in this dRR (i.e., the conclusions reached at Step 4 and 5 of the relevance assessment made at the EU-level are valid also with regard to the PEC_{gw} calculated for the GAP and groundwater scenarios considered in this dRR). M700F001 is not considered relevant according to the criteria laid down in the EC guidance document SANCO/221/2000 –rev.10.

A summary of the relevance assessment is given in Table 10.2-1 and the corresponding studies are listed in the corresponding sections.

Table 10.2-1: Summary of the relevance assessment for M700F001

	Assessment step		**Result of assessment**	
	STEP 1		Metabolite of no concern?	No
Quantification of groundwater contamination	STEP 2		Max PEC_{gw}	0.292 µg L^{-1}
			Based on	Spring cereals, $FOCUS_{gw}$ scenario: Jokioinen, model: FOCUS-PELMO 5.5.3, slow phase degradation
Hazard assessment	STEP 3	Stage 1	Biological activity comparable to the parent?	No
		Stage 2	Genotoxic properties of metabolite	Non-genotoxic
		Stage 3	Toxic properties of metabolite	Low acute toxicity
			Classification of parent	No classification relevant for groundwater metabolite assessment*
			Classification of metabolite	None
Consumer health risk assessment	STEP 4		Estimated consumer exposure via drinking water and other sources; threshold of concern approach	Acceptable [a]
	STEP 5		Refined risk assessment	Acceptable
			Predicted exposure (% of ADI)	<1% (EFSA, 2012)
			ADI based on	NOAEL of 250 mg kg^{-1} bw day^{-1} from the developmental toxicity study in rabbits with an AF of 1000 applied to account for the limited database (EFSA, 2012)

[a] According to EFSA (2012) the metabolite is non-relevant from the toxicological point of view according to the EC guidance document on the assessment of groundwater metabolites, as the studies provided sufficient evidence that this metabolite does not share the mode of action leading to carcinogenicity as observed with the parent fluxapyroxad. According to the recently concluded assessment by the ECHA's Risk Assessment Committee Fluxapyroxad does not require cancer classification in Carc.Cat.2; H351 (RAC Opinion, 2018). For details see end of chapter **10.2.3.3**.

10.2.1 STEP 1: Exclusion of degradation products of no concern

M700F001 does not meet the criteria for products of no concern as defined in step 1 of the guidance and therefore needs further assessment.

10.2.2 STEP 2: Quantification of potential groundwater contamination

PEC_{gw} calculations after leaching from soil for M700F001 were performed (see Part B, Section 8, Chapter 8.8.2). The uses for which concentrations of M700F001 were considered to exceed 0.1 µg L^{-1} are listed in Table 8.1-1 (GAP table). Details are given in Part B, Section 8, Chapter 8.8.

The maximum PEC_{gw} calculated for M700F001 in winter and spring cereals were above 0.1 µg L^{-1}, but below 0.75 µg L^{-1}.

10.2.3 STEP 3: Hazard assessment – identification of relevant metabolites

10.2.3.1 STEP 3, Stage 1: screening for biological activity

Fungicidal efficacy of Fluxapyroxad metabolite M700F001 was evaluated in glasshouse trials with nine major fungal pathogens representing the fungicide profile of fluxapyroxad (*Septoria tritici, Puccinia triticina, Pyrenophora teres, Rhynchosporium secalis, Phakopsora pachyrhizi, Alternaria solani, Sphaerotheca fuliginea, Botrytis cinerea* and *Venturia inaequalis*). None of the tested metabolites did provide significant efficacy against any of the fungal pathogens, while the parent compound fluxapyroxad provided very good control.

10.2.3.2 STEP 3, Stage 2: screening for genotoxicity

M700F001 was screened for genotoxic activity by the following data package of *in vitro* and *in vivo* genotoxicity studies: Ames test, *in vitro* gene mutation test with mammalian cells, and an *in vitro* chromosome aberration test and an *in vivo* micronucleus test. M700F001 was non-genotoxic as shown by a negative Ames test, negative gene mutation test with mammalian cells, negative chromosome aberration test *in vitro* and negative micronucleus test *in vivo*. M700F001 is considered not relevant and is further evaluated in Stage 3. The genotoxicity studies are evaluated in Part B, Section 6, studies referenced in DAR (Volume 3, Annex B.6.8).

10.2.3.3 STEP 3, Stage 3: screening for toxicity

M700F001 has been tested to determine its toxicological profile in acute and 28 and 90-day repeated dose toxicity studies in rats. Additionally, a developmental toxicity study in rabbits was conducted.

There are metabolic, structural, physicochemical and biological properties of M700F001 that indicate it is very unlikely that the metabolite will carry the toxicological properties of parent. M700F001 has been shown not to be genotoxic and consequently, there can be no concerns for non-threshold genotoxic carcinogenicity.

M700F001 is of low acute toxicity by the oral route. The toxicity of M700F001 has been investigated in a 28 and a 90-day dietary study in the rat. Both studies demonstrate that the toxicity of M700F001 is low. The 28-day dietary NOAEL for M700F001 is >1000 mg kg^{-1} bw day^{-1}, compared to 9-48 mg kg^{-1} bw day^{-1} for the parent, BAS 700 F. The 90-day oral NOAEL for M700F001 is >1000 mg kg^{-1} bw day^{-1} compared

to 6-7 mg kg^{-1} bw day^{-1} for the parent, BAS 700 F. These data show that M700F001 has considerably lower biological activity than BAS 700 F and does not pose a risk of carcinogenicity.

Therefore, M700F001 is considered not relevant and is further evaluated in Step 4. The toxicity studies are evaluated in Part B, Section 6, studies referenced in DAR (Volume 3, Annex B.6.8). The relevance assessment can be found in the DAR (Volume 3, Appendix 6) / EFSA conclusion.

10.2.4 STEP 4: Exposure assessment – threshold of concern approach

The potential exposure to M700F001 is >0.1 µg L^{-1} but below 0.75 µg L^{-1}. A further assessment in Step 5 is therefore not required.

Impact of the recent classification change of fluxapyroxad in relation to the toxicological relevance assessment of potential ground water metabolites of fluxapyroxad

Short-term toxicity testing of fluxapyroxad groundwater metabolites in 28-day and 90-day oral diet toxicity studies were performed because of the preliminary assessment of the parent fluxapyroxad to show limited evidence of carcinogenicity.

In the recently published Risk Assessment Committee (RAC) opinion on harmonised classification and labelling of fluxapyroxad (ECHA, 2018), RAC agreed that the so far applied cancer classification of fluxapyroxad (Canc. 2; H351) is not required. Furthermore, RAC unexpectedly considered based on incomplete information, that reduced pup weights observed in the rat 2-generation reproduction toxicity study are considered to reflect effects on or via lactation and therefore agreed on assigning the hazard phrase H362 “May cause harm to breast-fed children” to fluxapyroxad.

According to the Guidance Document on toxicological relevance assessment of groundwater metabolites SANCO 221/2000-rev10-final (25 February 2003), the R64 is notably not included in the list of parent R-phrases triggering endpoint-specific toxicological evaluations of the metabolites in groundwater (whereas R-phrases associated with impaired fertility /developmental toxicity classifications, i.e. R60, R61, R62, R63 are specifically listed in the SANCO Guidance Document).

Guidance on the toxicological relevance assessment of potential groundwater metabolites is provided in the SANCO Guidance Document 221/2000-rev10-final (25 February 2003):

> *“The guiding principle of the assessment is that a metabolite or degradation product is considered relevant, if there is reason to assume that it has comparable intrinsic properties as the active substance in terms of its biological target activity, or that it has certain toxicological properties that are considered severe (i.e. genotoxic, toxic to reproduction, carcinogenic, toxic or very toxic), unless demonstrated to the contrary.”*
>
> …
>
> *For parent active substances, which are classified for reproductive toxicity (any category with R60 R61, R62 or R63), it must be show by an appropriate test or convincing other evidence that the metabolite does not qualify for the same classification. Metabolites, which qualify for a classification of their reproductive toxicity (any category with R60 R61, R62 or R63) are considered to be“relevant”.*

The wording of the SANCO Guidance document indicates that, in the absence of evidence for developmental toxicity or impaired fertility (warranting classification), a mere classification of the parent molecule with R64 (corresponding to H362) does not constitute a sufficiently severe effect that would require an assessment of the groundwater metabolites for this endpoint. Strictly following the provisions of the SANCO Guidance Document, the new harmonised classification of fluxapyroxad does not constitute a trigger for further toxicological evaluation of fluxapyroxad metabolites in groundwater, other than their

assessment of the genotoxicity potential and their general toxicity (acute oral toxicity studies and 90-day dietary toxicity studies in rats, and prenatal developmental toxicity studies in rabbits are available for fluxapyroxad metabolites M750F001 and M750F002). All metabolite data were already evaluated at EU-level as part of the last peer-review process for authorisation of fluxapyroxad.

Conclusion

According to the SANCO Guidance Document SANCO 221/2000-rev10-final (25 February 2003, a classification for effects on or via lactation does not constitute a trigger for focused toxicological evaluation of fluxapyroxad metabolites regarding this endpoint, in the absence of an associated classification for impaired fertility or developmental toxicity. Therefore, a toxicological evaluation of fluxapyroxad metabolites for potential effects on or via lactation is not required.

10.3 Relevance assessment of M700F002

The relevance of the groundwater metabolite M700F002 has already been assessed and the assessment agreed at EU level (EFSA conclusion (2012 updated)), and the relevance assessment is applicable as well for the GAP and groundwater scenarios considered in this dRR (i.e., the conclusions reached at Step 4 and 5 of the relevance assessment made at the EU-level are valid also with regard to the PEC_{gw} calculated for the GAP and groundwater scenarios considered in this dRR). M700F002 is not considered relevant according to the criteria laid down in the EC guidance document SANCO/221/2000.

A summary of the relevance assessment is given in Table 10.3-1 and the corresponding studies are listed in the corresponding sections.

Table 10.3-1: Summary of the relevance assessment for M700F002

	Assessment step		**Result of assessment**	
	STEP 1		Metabolite of no concern?	No
Quantification of groundwater contamination	STEP 2		Max PEC_{gw}	2.838 µg L^{-1}
			Based on	Winter cereals, $FOCUS_{gw}$ scenario: Jokioinen, model: FOCUS-PEARL 4.4.4, fast phase degradation
Hazard assessment	STEP 3	Stage 1	Biological activity comparable to the parent?	No
		Stage 2	Genotoxic properties of metabolite	Non-genotoxic
		Stage 3	Toxic properties of metabolite;	Low acute toxicity
			Classification of parent	No classification relevant for groundwater metabolite assessment*
			Classification of metabolite	None
Consumer health risk assessment	STEP 4		Estimated consumer exposure via drinking water and other sources; threshold of concern approach	Not acceptable (>0.75 µg L^{-1}) [a]
	STEP 5		Refined risk assessment	Acceptable
			Predicted exposure (% of ADI)	<1% (EFSA, 2012)
			ADI based on	NOAEL of 300 mg kg^{-1} bw day^{-1} from the developmental toxicity study in rabbits with an AF of 1000 applied to account for the limited database (EFSA, 2012)

[a] According to EFSA (2012) the metabolite is non-relevant from the toxicological point of view according to the EC guidance document on the assessment of groundwater metabolites, as the studies provided sufficient evidence that this metabolite does not give rise to concern.
* Fluxapyroxad does not require cancer classification in Carc.Cat.2; H351 (RAC Opinion, 2018). For details see additional information provided at the end of chapter **10.2.3.3**.

10.3.1 STEP 1: Exclusion of degradation products of no concerns

M700F002 does not meet the criteria for products of no concern as defined in step 1 of the guidance and

therefore needs further assessment.

10.3.2 STEP 2: Quantification of potential groundwater contamination

PEC_{gw} calculations after leaching from soil for M700F002 were performed (see Part B, Section 8, Chapter 8.8.2). The uses for which concentrations of M700F002 were considered to exceed 0.1 µg L^{-1} are listed in Table 8.1-1 (GAP table). Details are given in Part B, Section 8, Chapter 8.8.

The maximum PEC_{gw} calculated for M700F002 in winter and spring cereals were above 0.75 µg L^{-1}, but below 10 µg L^{-1}.

10.3.3 STEP 3: Hazard assessment – identification of relevant metabolites

10.3.3.1 STEP 3, Stage 1: screening for biological activity

Fungicidal efficacy of Fluxapyroxad metabolite M700F002 was evaluated in glasshouse trials with nine major fungal pathogens representing the fungicide profile of fluxapyroxad (*Septoria tritici, Puccinia triticina, Pyrenophora teres, Rhynchosporium secalis, Phakopsora pachyrhizi, Alternaria solani, Sphaerotheca fuliginea, Botrytis cinerea* and *Venturia inaequalis*). None of the tested metabolites did provide significant efficacy against any of the fungal pathogens, while the parent compound fluxapyroxad provided very good control.

10.3.3.2 STEP 3, Stage 2: screening for genotoxicity

M700F002 was screened for genotoxic activity by the following data package of *in vitro* and *in vivo* genotoxicity studies: Ames test, *in vitro* gene mutation test with mammalian cells, and an *in vitro* chromosome aberration test and an *in vivo* micronucleus test. M700F002 was non-genotoxic as shown by a negative Ames test, negative gene mutation test with mammalian cells, negative chromosome aberration test *in vitro* and negative micronucleus test *in vivo*. M700F002 is considered not relevant and is further evaluated in Stage 3. The genotoxicity studies are evaluated in Part B, Section 6, studies referenced in DAR (Volume 3, Annex B.6.8).

10.3.3.3 STEP 3, Stage 3: screening for toxicity

The parent, BAS 700 F, to M700F002 is classified as a carcinogen in category 2. M700F002 has therefore been tested to determine its toxicological profile in acute and 28 and 90-day repeated dose toxicity studies in rats in accordance with the EC guidance document SANCO/221/2000 –rev.10. Additionally, a developmental toxicity study in rabbits was conducted.

There are metabolic, structural, physicochemical and biological properties of M700F002 that indicate it is very unlikely that the metabolite will carry the toxicological properties of parent. M700F002 has been shown not to be genotoxic and consequently, there can be no concerns for non-threshold genotoxic carcinogenicity.

M700F002 is of low acute toxicity by the oral route. The toxicity of M700F002 has been investigated in a 28 and a 90-day dietary study in the rat. Both studies demonstrate that the toxicity of M700F002 is low. The 28-day dietary NOAEL for M700F002 is >1000 mg kg^{-1} bw day^{-1}, compared to 9-48 mg kg^{-1} bw day^{-1} for the parent, BAS 700 F. The 90-day oral NOAEL for M700F002 is >1000 mg kg^{-1} bw day^{-1} compared to 6-7 mg kg^{-1} bw day^{-1} for the parent, BAS 700 F. These data show that M700F002 has considerably lower biological activity and does not pose a risk of carcinogenicity.

Therefore, M700F002 is considered not relevant and is further evaluated in Step 4. The toxicity studies are evaluated in Part B, Section 6, studies referenced in DAR (Volume 3, Annex B.6.8). The relevance assessment can be found in the DAR (Volume 3, Appendix 6) / EFSA conclusion.

10.3.4 STEP 4: Exposure assessment – threshold of concern approach

The potential exposure to M700F002 is >0.75 µg L^{-1} but <10 µg L^{-1}. A further assessment in Step 5 is required.

10.3.5 STEP 5: Refined risk assessment

M700F002 has maximum PEC_{gw} >0.75 µg L^{-1}, but below 10 µg L^{-1}. A refined assessment of the potential toxicological significance including the selected ADI is presented here.

According to EFSA (2012) the additional intake through drinking water of M700F002 is estimated to be <1% of the total ADI. The ADI is 0.3 mg kg^{-1} bw day^{-1}, based on the NOAEL of 300 mg kg^{-1} bw day^{-1} from the developmental toxicity study in rabbits with an AF of 1000 applied to account for the limited database available (no long-term, multigeneration or rat developmental toxicity study available).

Even when considering a theoretical drinking water concentration of 10 µg L^{-1} for M700F002 and assuming a life-long daily intake of 2 L drinking water, the ADI utilization for the consumer for M700F002 residues is 0.1% ($ADI_{M700F002}$ = 0.3 mg kg^{-1}); therefore, any risk for consumers via drinking water can be excluded.

10.4 Relevance assessment of R234886

Summary:

The groundwater metabolite R234886 is considered as relevant according to the criteria laid down in the EC guidance document SANCO/221/2000 –rev.10. A summary of the relevance assessment for R234886 is given in Table 10.4-1. Studies supporting PEC_{GW} data are evaluated in Section 8 (Environmental fate and behaviour), the genotoxicity studies are evaluated in Section 6 (Mammalian Toxicology); the data on biological activity are evaluated in Appendix 2 of this Section.

As R234886 does not demonstrate exceedances of the threshold of 0.75 µg/L in any FOCUS scenarios, it therefore does not require a refined risk assessment.

Table 10.4-1: Summary of the relevance assessment for R234886

	Assessment step		**Result of assessment**	
	STEP 1		Metabolite of no concern?	no
Quantification of groundwater contamination	STEP 2		Max PEC_{GW}	0.513 µg/L
			Based on	FOCUS-PEARL (v4.4.4), application to spring cereals, scenario Hamburg (Chapter 8.8.2, Part B Section 8)
Hazard assessment	STEP 3	Stage 1	Biological activity comparable to the parent?	No
		Stage 2	Genotoxic properties of metabolite	Non genotoxic
		Stage 3	Toxic properties of metabolite:	Acute oral toxicity: > 5000 mg/kg bw
			Classification of parent	H331
			Classification of metabolite	None
Consumer health risk assessment	STEP 4		Estimated consumer exposure via drinking water and other sources; threshold of concern approach	Acceptable (<0.75 µg/L)
	STEP 5		Refined risk assessment	NA
			Predicted exposure (% of ADI)	NA
			ADI based on	NA

NA = not applicable

10.4.1 STEP 1: Exclusion of degradation products of no concern

R234886 does not meet the criteria for products of no concern as defined in step 1 of the guidance and therefore needs further assessment.

10.4.2 STEP 2: Quantification of potential groundwater contamination

PEC_{GW} calculations after leaching from soil for R234886 were performed considering the proposed use of BAS 736 00 F on cereals. The ground water concentrations of R234886 was predicted to exceed 0.1 µg/L (but not 0.75 µg/L) in a number of FOCUS scenarios. Details are given in Part B, Section 8, chapter 8.8.2. Further assessment is therefore required to determine its relevance with regard to potential for groundwater contamination.

10.4.3 STEP 3: Hazard assessment – identification of relevant metabolites

10.4.3.1 STEP 3, Stage 1: screening for biological activity

The study on biological activity performed on R234886 has been previously reviewed under Council Directive 91/414/EEC (EFSA Journal (2010) 8(4), 1542).

Based on evidence from a fungicide screen, R234886 did not show any fungicidal activity when applied at rates known to be effective for parent azoxystrobin. Therefore R234886 is not considered to be biologically active.

Furthermore the available data indicate that R234886 is considerably less ecotoxic than the parent azoxystrobin, confirming the difference in activity between the two substances.

10.4.3.2 STEP 3, Stage 2: screening for genotoxicity

A study on genotoxicity performed on R234886 has been previously reviewed under Council Directive 91/414/EEC (EFSA Journal (2010) 8(4), 1542).

The mutagenic potential of R234886 was evaluated in a bacterial mutagenicity assay over a range of concentrations using four strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and two strains of *Escherichia coli* (WP2 (pKM101) and WP2 *uvrA* (pKM101)) in the presence and absence of a rat liver-derived metabolic activation system (S9-mix).

Under the conditions of this assay, R234886 gave a negative, i.e. non-mutagenic, response in *S.typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E.coli* strains WP2 (pKM101) and WP2 *uvrA* (pKM101) in both the presence and absence of S9-mix.

Further, R234886 is the acid metabolite of azoxystrobin and has been identified in the plasma of rats and rabbits following administration of parent azoxystrobin. The glucuronide conjugate of R234886 (metabolite V) is found at high levels, i.e. up to 29% of the dose in bile, in rats dosed with parent azoxystrobin (Azoxystrobin DAR Volume 3, Annex B.6).

Gene mutation tests with mammalian cells and chromosome aberration tests have not been conducted with R234886 because there was deemed to be adequate exposure to R234886 in the *in vivo* genotoxicity studies conducted with parent azoxystrobin. Azoxystrobin was found to be negative in the established *in vivo* assays for chromosomal damage (i.e. clastogenicity) and for interaction with the DNA (UDS test for DNA damage and repair) and furthermore, chronic studies have not shown any evidence of carcinogenicity in mouse and rat.

Based on evidence from a bacterial mutagenicity study with R234886 and *in vivo* genotoxicity tests with the parent azoxystrobin, it is concluded that metabolite R234886 is not genotoxic and further testing is not required.

10.4.3.3 STEP 3, Stage 3: screening for toxicity

Extensive toxicity testing of the active substance has been carried out and the results are described in detail in the EFSA Journal 2010; 8 (4): 1542.

The toxicity of azoxystrobin and R234886 has been evaluated in acute oral toxicity tests. The results of these studies indicate that neither azoxystrobin nor R234886 exhibits any toxicity at doses up to 5000 mg/kg bw. Toxicity tests with azoxystrobin are considered representative of the potential effects of R234886 because R234886 is the acid metabolite of azoxystrobin and has been identified in the plasma of rats and rabbits following administration of parent azoxystrobin. The glucuronide conjugate of R234886 (metabolite V) is found at high levels, i.e. up to 29% of the dose in bile, in rats dosed with parent azoxystrobin (Azoxystrobin DAR Volume 3, Annex B.6). The toxicological properties of azoxystrobin have been thoroughly evaluated and azoxystrobin has been shown to have low acute toxicity, is not genotoxic in vivo and showed no evidence of carcinogencity in either the rat or mouse when dosed for up to two years. Furthermore, azoxystrobin is not teratogenic or reprotoxic and does not exhibit evidence of neurotoxicity in any of the toxicity studies conducted. Therefore, the active substance does neither fulfil the criteria for classification and labelling for reproductive or developmental toxicity nor for carcinogenicity.

The parent compound azoxystrobin is not classified for reproductive toxicity, mutagenicity or carcinogenic properties, *i.e.* is not classified with either the signal word Danger or Warning, the pictogram GHS08, or with the hazard phrases; H340, H341, H350, H351, H360, H361 or H362. Consequently, toxicity testing with R234886 is not required based on these criteria.
The active substance azoxystrobin fulfils the criteria for classification and labelling as ‘toxic’ with regard to inhalation toxicity (GHS06, Signal word; Danger, H331), however for groundwater metabolites inhalation toxicity is of limited relevance.

In the case of R234886 it has to be considered that this metabolite occurs not only in groundwater but is also generated in mammalian metabolism in a considerable fraction. Therefore, EFSA decided during the Peer Review process for the active substance, that non-relevance of the metabolite R234886 can be demonstrated based on the available data. In the EFSA conclusion on the pesticides peer review of the active substance azoxystrobin, the metabolite R234886 is classified as not relevant for groundwater (see EFSA Journal 2010; 8(4):1542). Furthermore, data from ecotoxicity tests also indicate that R234886 is considerably less toxic to aquatic and soil organisms than the parent azoxystrobin.

10.4.4 STEP 4: Exposure assessment – threshold of concern approach

The potential exposure to metabolite R234886 is > 0.1 µg/L but < 0.75 µg/L. Therefore, a further assessment in Step 5 is not required.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

There are no studies submitted with this section.

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

BAS 736 00 F is a new product, no product data have been evaluated previously.

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Appendix 2 Additional information

The following study header is written in *italics* because the study was evaluated previously (DAR Addendum 2014).

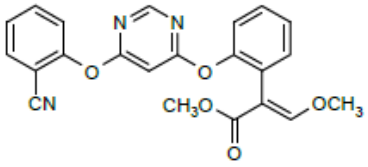
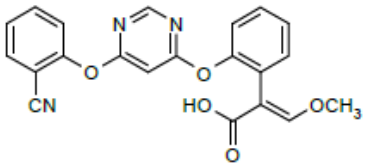
Reference:	*CP 13/1*
Report	*Azoxystrobin - Metabolite R234886: Evaluation of intrinsic fungicidal activity,* *Anonymous, 2021* *report No TMJ5077B* *TMJ5077B* *Authority registration No*
Guideline(s):	*none*
Deviations:	*Not applicable*
GLP:	*not conducted under GLP/Officially recognised testing facilities; not required for this study type*
Acceptability:	*Yes* *previously submitted, evaluated and accepted (DAR Addendum 2014 : the study is included in the 'references relied upon' list.*

Introduction

The fungicidal activity of R234886 (a metabolite of azoxystrobin) was evaluated against a range of representative fungal pathogens in a screen performed at Syngenta Crop Protection, Münchwilen AG, Research Biology Centre, Schaffhauserstrasse, 4332 Stein, Switzerland.

The azoxystrobin metabolite R234886 is formed in the soil at a maximum formation rate of 20% in laboratory studies. It is therefore necessary to demonstrate that the metabolite does not have the same level of intrinsic fungicidal activity as azoxystrobin.

Table A 1: Structure of azoxystrobin and the azoxystrobin metabolite R234886

 Azoxystrobin	Molecular weight 403.4 $C_{22}H_{17}N_3O_4$
 R234886	Molecular Weight 389.37 $C_{21}H_{15}N_3O_5$

Materials and Methods

The activity of R234886 and azoxystrobin were evaluated against a representative foliar pathogen from each of the major classes of phytopathogenic fungi.

Oomycete - *Phytophthora infestans* (late blight) on tomato
Basidiomycete - *Puccinia recondita f.sp. triticina* (brown rust) on wheat
Ascomycete - *Cercospora arachidicola* (early leaf spot) on peanut
Deuteromycete - *Alternaria solani* (early blight) on potato

The pathogens were selected on the basis that azoxystrobin demonstrated good levels of activity against them. An initial rate setting exercise was conducted to select the treatment rates for use in the final study. Details of this rate setting exercise are not reported in this study.

Prior to treatment application a stock solution was made up by dissolving technical material in acetone and adding an aliquot to a pre-determined volume of the blank experimental formulation IF-50 to obtain the desired treatment concentration. The treatment dilutions were made using a dilution machine (prototype developed by Caromatic using Kloehn-Diluters). The effect of azoxystrobin and R234886 on the target fungi were compared to an untreated control, a formulation blank (negative control) and a commercial standard. For each disease, a leading commercial standard was prepared in IF-50 and used as the positive control. For *P. infestans, P. recondita* and *C. arachidicola*, the commercial standards were metalaxyl, epoxiconazole and difenoconazole respectively. For *A. solani*, azoxystrobin was used as the commercial standard with a second replicate set of test plants being established. The experimental formulation blank IF-50 was used as a negative control for each of the four pathogens. The test treatment and positive controls were applied at a minimum of five rates and four replicate treatments were established for each test treatment and control. For each pathogen, 20 untreated control pots were inoculated in order to establish the level of disease for the untreated control.

All test treatments were applied prophylatically to the test plants using an application machine (prototype developed by Caromatic; turntable, air supported spraying from 2 nozzles). The exact timing of applications and assessments, together with the environmental conditions, were optimised for each pathogen, details of which are presented below.

Phytophthora infestans (potato late blight)

Three-week-old potato plants cv. Bintje were treated with the test compounds in a spray chamber. Two days after the treatment application the plants were inoculated by spraying a sporangial suspension (60,000 sporangia/mL) on to the test plants. After an incubation period of 4 days at 18o C and 100 % r.h. in a growth chamber, the percentage leaf area covered by disease was assessed.

Puccinia recondita (wheat brown rust)

One week old wheat plants cv. Arina were treated with the test compounds in a spray chamber. One day after application, the wheat plants were inoculated by spraying a spore suspension (70,000 uredo-spores/mL) on to the test plants. The plants were incubated for 24 hours at 20ºC and 95% r.h. before being transferred to a glasshouse for the 10 day incubation period (plants were kept for 10 days 20° C / 18° C (day/night) and 60% r.h. in a glasshouse). The percentage leaf area covered by disease was assessed 11 days after inoculation.

Cercospora arachidicola (Mycosphaerella arachidis) (peanut early leaf spot)

Three-week-old peanut plants cv. Georgia Green were treated with the test compounds in a spray chamber. One day after application, the lower leaf surface was sprayed with a spore suspension (400,000 spores/mL). The plants were incubated for four days under a plastic hood at 23° C and 100% r. h. For the remainder of the study, the plants were kept at 23° C / 20° C (day/night) and 70% r.h. in a greenhouse. The percentage leaf area covered by disease was assessed 13 days after inoculation.

Alternaria solani (tomato early blight)

Four-week-old tomato plants cv. Roter Gnom were treated with the test compounds in a spray chamber. Two days after treatment application tomato the plants were inoculated by spraying a spore suspension (6,000 spores/mL) on the test plants. The treated and inoculated plants were incubated in a glasshouse for 3 days at 22/18o C and 95% r. h., after which time the percentage leaf area covered by disease was assessed. In this component of the test the commercial standard used was a pre-formulated azoxystrobin (solo) product as this is the leading standard for tomato early blight control.

Results

A summary of the results is presented in the table below. Dose response graphs are presented in the figures section. The percentage disease control (efficacy) was calculated by comparing the percentage disease (leaf coverage) in the test treatment to the percentage disease in the untreated control using the equation below.

$$\%\ Efficacy = 1 - \left(\frac{\%\ Disease\ in\ test\ treatment}{\%\ Disease\ in\ untreated}\right) \times 100$$

Table A 2: Fungicidal activity of azoxystrobin and R234886

Test treatment	Rate (ppm)	Target pathogen							
		Phytophthora infestans		*Puccinia recondita*		*Cercosporaarachidicola*		*Alternaria solani*	
		% Disease	% Disease control	% Disease	% Disease control	% Disease	% Disease control	% Disease	% Disease control
Untreated		80	-	80	-	80	-	79	-
IF-50		77.5	3.1	80	0	80	0	80	0
Azoxystrobin	6.0	25.0	68.8	N.D.	N.D.	N.D.	N.D.	15	81.0
	2.0	27.5	65.6	20	75.0	75	6.3	30	62.0
	0.6	67.5	15.6	42.5	46.9	72.5	9.4	45	43.0
	0.2	77.5	3.1	77.5	3.1	80	0.0	70	11.4
	0.06	77.5	3.1	80	0.0	80	0.0	75	5.1
	0.02	N.D.	N.D.	80	0.0	80	0.0	N.D.	N.D.
R234886	6.0	80	0.0	N.D.	N.D.	N.D.	N.D.	80	0.0
	2.0	80	0.0	80	0.0	77.5	3.1	80	0.0
	0.6	80	0.0	80	0.0	80	0.0	80	0.0
	0.2	80	0.0	80	0.0	80	0.0	80	0.0
	0.06	80	0.0	80	0.0	80	0.0	80	0.0
	0.02	N.D.	N.D.	80	0.0	80	0.0	N.D.	N.D.
Commercialstandard [a]	20.0	0	100	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	6.0	0	100	N.D.	N.D.	0	100	6.25	92.1
	2.0	0.5	99.4	4.25	94.7	3	96.3	5	93.7
	0.6	6	95.5	17.5	78.1	6.75	91.6	15	81.0
	0.2	57.5	28.1	72.5	9.4	72.5	9.4	17.5	77.8
	0.06	75	6.3	80	0.0	72.5	9.4	45	43.0
	0.02	N.D.	N.D.	80	0.0	70	12.5	62.5	20.9

[a] The commercial standards used were: *P. infestans*-metalaxyl, *P. recondita*-epoxiconazole, *C. arachidicola*-difenoconazole and *A. solani*-azoxystrobin.
ND not determined

Overall the results indicate high levels of disease in the untreated controls and the blank experimental formulation IF-50 exhibiting no efficacy against any of the test species. The commercial standards gave good disease control with approximately 100% efficacy being observed at the highest treatment rates and efficacy decreasing in line with a reduction in the treatment rate.

Azoxystrobin displayed good efficacy against *P. infestans*, *P. recondita* and *A. solani*. However, azoxystrobin did not give appreciable disease control against *C. arachidicola*. This observation is a little surprising as azoxystrobin normally exhibits good efficacy against *C. arachidicola* and is most likely due to azoxystrobin being applied at slightly too low a rate to provide disease control.

The azoxystrobin metabolite R234886 did not give control of any of the pathogens at any of the rates tested indicating that it does not possess the same intrinsic fungicidal activity as azoxystrobin.

Conclusion

The azoxystrobin metabolite R234886 demonstrated no fungicidal activity against a range of foliar pathogens against which azoxystrobin gave good levels of disease control confirming that it has no intrinsic fungicidal activity.

Figures

Figure A-1: Control of *Phytophthora infestans* on potato.

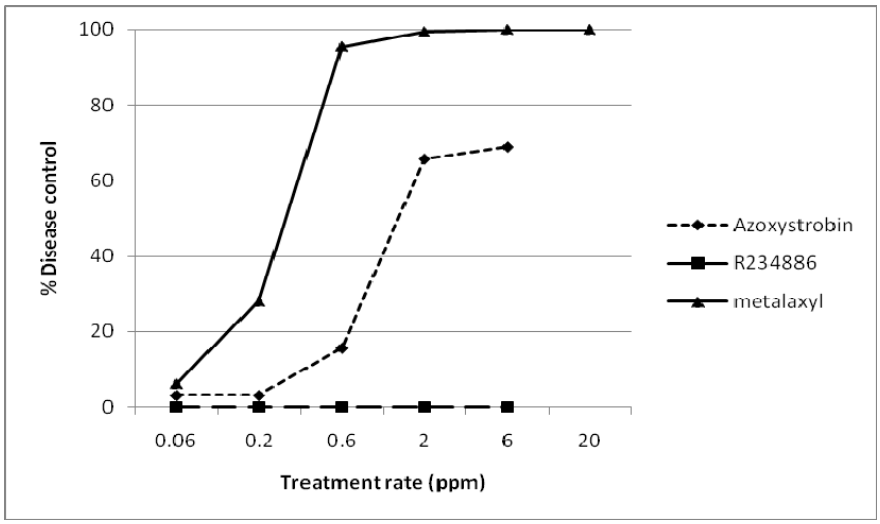


Figure A-2: Control of *Puccinia recondita* on wheat.

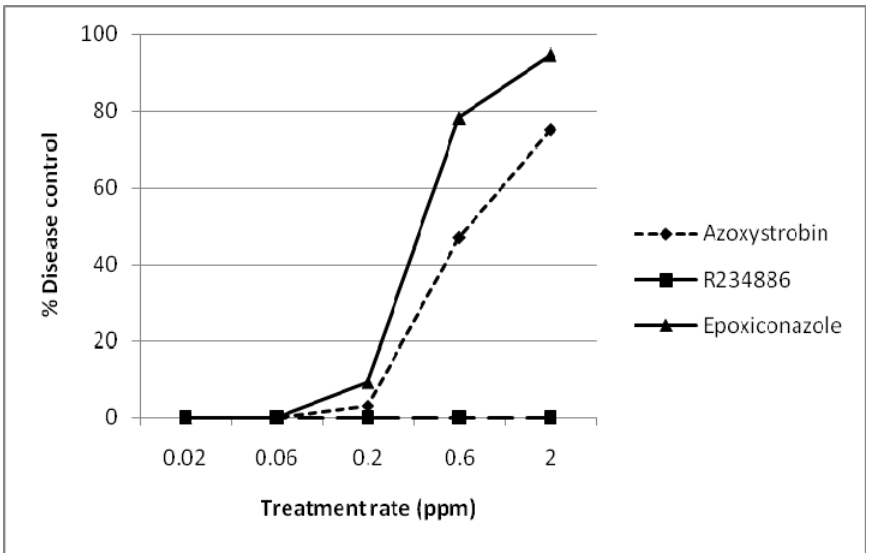


Figure A-3: Control of *Cercospora arachidicola* on peanut.

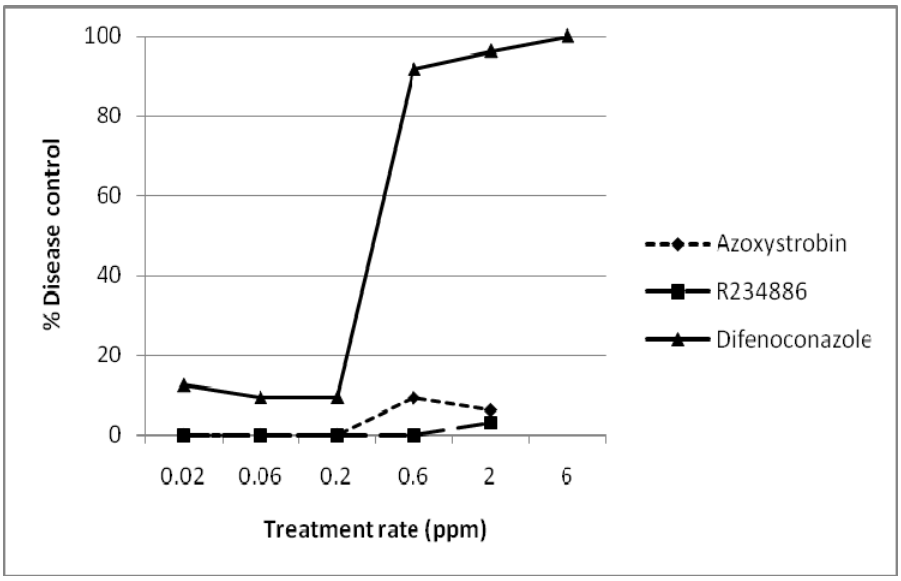


Figure A-4: Control of *Alternaria solani* on tomato.