REGISTRATION REPORT

Part B

Section 10

Assessment of the relevance of metabolites in groundwater

Detailed summary of the risk assessment

Product code: 102000012886

Product name(s): Fluopyram + trifloxystrobin SC 500

Active substance(s): (250 + 250 g/L)

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(Re-Authorisation)

Applicant: Bayer Crop Science Division

Submission date: 30/06/2020

MS Finalisation date: July 2021 (initial Core Assessment)

March 2022 (final Core Assessment)

Version history

When	What
June 2020	Applicant initial dRR
July 2021	Initial assessment by the zRMS The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~.
March 2022	Final report (Core Assessment after the commenting period) Additional information/assessments included by the zRMS in the report in response to comments recieved from the cMS and the Applicant are highlighted in yellow, while not agreed use pattern is ~~struck through and shaded~~.

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Reviewer comments:

This part of dossier has been submitted to support re-authorisation of the plant protection product Luna Sensation 500SC/FLU+TFS SC 500 according art. 43 of 1107/2009.
Document summarizes data related to the relevance of metabolites in groundwater. Information contained in dRR Part B10 has been reviewed for the purposes of ongoing registration and considered as sufficient and appropriate for risk assessment.
~~ZRMS refinement has been added to STEP 5 for the following trifloxystrobin methabolites (CGA 321113, NOA 413161, NOA 413163) reflecting EFSA recommendation (*EFSA Scientific Committee; Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579. [32 pp.] doi:10.2903/j.efsa.2012.2579*) regarding adult and child bodyweight for dietary exposure assessment.~~
Reflecting the comments of cMS AT and NL regarding WHO drinking water guidance which is taken into account for the refined risk assessment, leading to higher values covering the worst-case, ZRMS revised correction in STEP 5.

The product fluopyram + trifloxystrobin SC 500 (250+250 g/L) (FLU + TFS SC 500 / Product Code 102000012886) was not the representative formulation during the renewal of approval of trifloxystrobin. All data and information assessed during the EU re-evaluation of trifloxystrobin is considered EU peer-reviewed data.

10 Relevance of metabolites in groundwater

10.1 General information

Trifloxystrobin

Three metabolites are predicted to occur in groundwater at concentrations above 0.1 µg/L (refer to Chapter 8.8 in the Part B section 8 Environmental Fate of the Core Assessment). Assessment of the relevance of metabolite 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 the table below. 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 chapter 8.8 of the dRR Part B, Section 8 (Environmental fate and behaviour).

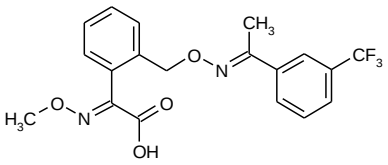
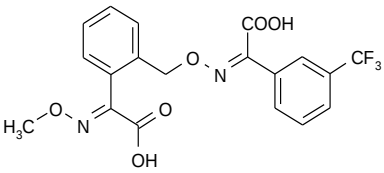
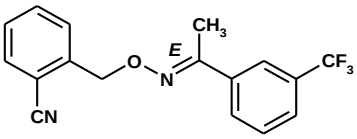
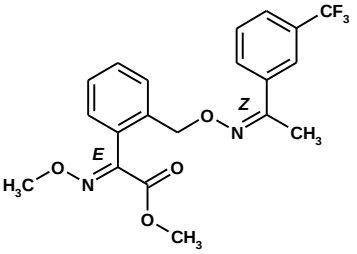
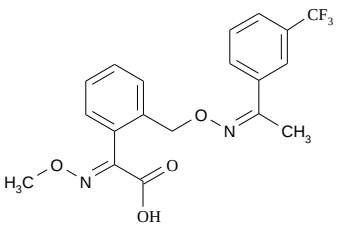
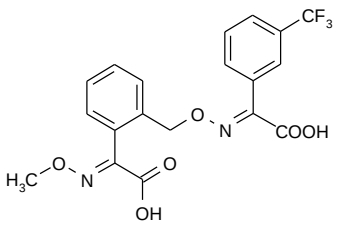
Fluopyram

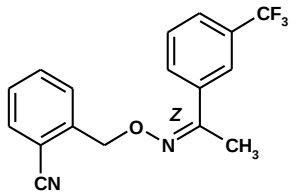
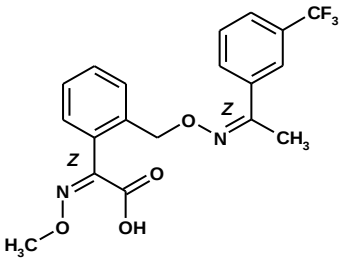
For the Renewal of Authorisations according to Article 43 of Regulation (EC) No 1107/2009, the following guidance is given in the Document SANCO/2010/13170 for products containing two or more active substances:

- “when the 1st substance is renewed- there is no need to evaluate data related to the 2nd substance”
- “once the 2nd substance is renewed- there is no need to evaluate data related to the 1st substance because this has already been performed in the frame of the re-authorisation of the PPP following the renewal of the 1st active substance”
- “Where necessary a combitox assessment should be performed.”

In consequence, metabolites of fluopyram are not considered in the risk assessment as this would be out of scope of SANCO/2010/13170

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

Name of active substance	Metabolite name and code	Structural/molecular formula	Trigger for relevance assessment	
Trifloxystrobin (CGA 279202)	**CGA 321113 (EE-isomer)**		Relevance assessment: Max PEC_{gw} Based on:	Yes 2.042 µg/L FOCUS PEARL for scenario Hamburg, chokeberry, elderberry, tree nursery (modelling crop: Apples II (late)), 2 × 200 g a.s./ha
	NOA 413161 (ZE-isomer)		Relevance assessment: Max PEC_{gw} Based on:	Yes 7.278 µg/L FOCUS PEARL for scenario Jokioinen, Lambs lettuce, lettuce, rocket salad (modelling crop: Cabbage II (early)), 2 × 200 g a.s./ha
	CGA 357276 (E-isomer)		Relevance assessment: Max PEC_{gw} Based on:	No < 0.1 µg/L Calculations by using FOCUS PEARL & PELMO for all scenarios and all crops 2 × 200 g a.s./ha
	CGA 357261 (TFS ZE-isomer)		Relevance assessment: Max PEC_{gw} Based on:	No < 0.1 µg/L Calculations by using FOCUS PEARL & PELMO for all scenarios and all crops 2 × 200 g a.s./ha
	CGA 373466* (ZE-isomer)		Relevance assessment: Max PEC_{gw} Based on:	No < 0.1 µg/L Calculations by using FOCUS PEARL & PELMO for all scenarios and all crops 2 × 200 g a.s./ha
	NOA 413163 (EE-isomer)**		Relevance assessment: Max PEC_{gw} Based on:	Yes 9.894 µg/L FOCUS PEARL for scenario Jokioinen, Apples II (early), 2 × 200 g a.s./ha

Name of active substance	Metabolite name and code	Structural/molecular formula	Trigger for relevance assessment	
	NOA 409480 (Z-isomer)		Relevance assessment: Max PEC_{gw} Based on:	No < 0.1µg/L Calculations by using FOCUS PEARL & PELMO for all scenarios and all crops, 2 × 200 g a.s./ha
	CGA 381318 (ZZ-isomer)		Relevance assessment: Max PEC_{gw} Based on:	No < 0.1µg/L Calculations by using FOCUS PEARL & PELMO for all scenarios and all crops, 2 × 200 g a.s./ha

* PECgw values for CGA 373466 presented in section B8 exceed the trigger of 0.1 µg/L for chokeberry, elderberry, tree nursery (modelling crop: Apples II, 2 × 200 g a.s./ha). However, the concerned FOCUS scenarios are either not relevant for the corresponding member state (e.g. NLD) or a GAP change is necessary as other metabolites (NOA 413163, see below) exceed the trigger value of 10 µg/l. The proposed change of the GAP reults in a PECgw below 0.1 µg/L for CGA 373466. The PEC gw values shown in this section for CGA 373466 were chosen based on the assumption that the proposed GAP change is acceptable,
** PEC_{gw} values for NOA 413163 presented in section B8 exceed the trigger of 10 µg/L for chokeberry, elderberry, tree nursery (modelling crop: Apples II, 2 × 200 g a.s./ha) and lambs lettuce, lettuce and rocket salad (modelling crop: Cabbage II, 2 × 200 g a.s./ha) in some FOCUS scenarios. Please note, in case the concerned FOCUS scenario is relevant for the corresponding member state and an exceedance of trigger of 10 µg/L is not allowed (e.g. use 52 in POL), a GAP change was proposed directly in section B8. The maximum PECgw values shown in this section for NOA 413163 were chosen based on the assumption that the proposed GAP change is acceptable.

10.2 Relevance assessment of metabolite CGA 321113

The relevance of the groundwater metabolite CGA 321113 has already been assessed and the assessment agreed at EU level (see EFSA Journal 2017;15(10):4989), but the relevance assessment is not applicable 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 not valid with regard to the PECgw calculated for the GAP and groundwater scenarios considered in this dRR). Therefore, the assessment and conclusions are presented here.

Trifloxystrobin metabolite **CGA 321113** 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 below.

Table 10.2-1: Summary of the relevance assessment for metabolite CGA 321113

	Assessment step		Result of assessment	
	STEP 1		Metabolite of no concern?	No
Quantification of ground water contamination	STEP 2		Max PEC_{gw}	2.042 µg/L
			Based on	FOCUS PEARL for scenario Hamburg, chokeberry, elderberry, tree nursery (modelling crop: Apples II (late)), 2 × 200 g a.s./ha
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 oral toxicity to rats (LD_{50} > 2000 mg/kg bw), Comparative in vitro experiment: CGA 321113 caused significantly less inhibition of mitochondrial respiration than trifloxystrobin, CGA 321113 is contributing to >10% (in bile of male and female rats) of absorbed dose of TFS in the rat ADME study. Therefore, CGA 321113 is considered as characterized by the toxicity studies performed with parent compound.
			Classification of parent	Skin Sens. 1, H317 Effects on or via lactation, H362
			Classification of metabolite	Not classified
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)
	STEP 5		Refined risk assessment	Acceptable
			Predicted exposure (% of ADI)	Intake by means of drinking water: 0.306% ADI (infant, 5 kg) 0.204 (child, 10 ~~12~~ kg) ~~0.17~~ % ADI 0.068 (adult, 60 ~~70~~ kg) ~~0.058~~ % ADI Maximum intake by means of food of plant and animal origin: 50% (NL toddler; assuming all residues consist of CGA 321113) Total intake: 50% of ADI
			ADI based on	0.1 mg/kg bw/d (ADI for parent trifloxystrobin according to EFSA Journal 2017;15(10):4989)

10.2.1 STEP 1: Exclusion of degradation products of no concern

The metabolite does not meet the criteria for products of no concern as defined in step 1 of the guidance and therefore they need further assessment.

It cannot be excluded as a product of no concern as they are not:

- CO2 or an inorganic compound, not containing a heavy metal;
- an organic compound of aliphatic structure, with a chain length of 4 or less, consisting 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;
- 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.

10.2.2 STEP 2: Quantification of potential groundwater contamination

PEC_{gw} calculations after leaching from soil were performed (Part B, Section 8 for details data). The uses for which metabolite concentrations were considered to exceed 0.1 µg/L are listed in the table above. **Therefore further assessment of the relevance of the metabolite is required** in terms of biological, genotoxicological and toxicological activity according to EU Guidance Document SANCO/221/2000, rev. 10 (2003).

10.2.3 STEP 3: Hazard assessment – identification of relevant metabolites

10.2.3.1 STEP 3, Stage 1: screening for biological activity

Tests were conducted to investigate the biological activity of the lysimeter metabolite CGA 321113 (EE). A short summary of the results given in Annex II, point 3 of trifloxystrobin (M-069325-01-1)[1] is presented in the following paragraph:

"It could be shown that both mono- and both bis-acids have lost most of their biological activity compared to the active substance CGA 279202. In two separate in-vivo tests it could be shown that the acid metabolite of CGA 279202, CGA 321113 is by a factor of thousand less biological active than the parent. From the four metabolites tested, CGA 321113 showed the highest biological activity. CGA 373466 (ZE-isomer of CGA 321113) as well as the bis-acids are again a factor of ten less active than the monoacid CGA 321113. The effect that the EE isomer is biological more active than the ZE could also be shown for the parent compound in in-vivo tests, thereby the biological activity of CGA 279202 and its isomers decreased in the following order: EE > EZ ≥ ZE (ZZ was not active). In these in-vivo tests no biological activity was detected for the metabolite CGA 321113."

The Guidance Document states that metabolites with a biological activity comparable or higher than the parent are considered as relevant. This metabolite is considered not relevant and is further evaluated in Step 3, stage 2.

10.2.3.2 STEP 3, Stage 2: screening for genotoxicity

CGA 321113 was screened for genotoxic activity by various in vitro and in vivo genotoxicity studies including: Ames test, gene mutation test with mammalian cells, a chromosome aberration test, and an in vivo micronucleus test. CGA 321113 was non-genotoxic (please refer to RAR, Appendix A: List of Endpoints: "Unlikely to be genotoxic in vivo") as shown by a negative Ames test, negative gene mutation test with mammalian cells, positive chromosome aberration test, negative in vivo micronucleus test and a negative unscheduled DNA synthesis assay in vivo.

Thus, the metabolite is considered to be 'non-relevant' regarding genotoxicity and is further evaluated in Step 3.

10.2.3.3 STEP 3, Stage 3: screening for toxicity

The parent compound, trifloxystrobin, is classified for health effects in accordance with CLP Regulation Annex VI, Part 3 List of harmonized classifications (EC-Regulation 790/2009) only for skin sensitization, Category 1 (H317 May cause an allergic skin reaction). In addition, following the ECHA/RAC 50 meeting for trifloxystrobin, the Committee agreed to classify trifloxystrobin for effects through or via lactation (may cause harm to breast-fed children, Lact.; H362).

The parent, trifloxystrobin, to **CGA 321113** is not classified as acutely or chronically toxic or very toxic / for reproductive toxicity / as a carcinogen in category 1 or 2 (or corresponding classification in accordance to CLP 1272/2008). There are no reasons to expect that CGA 321113 may be toxic or highly toxic.

[1] Ohs, P., Tier II, IIA, 3: Data on application, Report No.: MO-02-008756, Edition Number: M-069325-01-1, 2002. Can be made available on request.

CGA 321113 is of low acute oral toxicity to rats (LD_{50} > 2000 mg/kg bw), in a comparative in vitro experiment CGA 321113 caused significantly (900-1000 times) less inhibition of mitochondrial respiration than trifloxystrobin. Since significant inhibition of cellular respiration is likely to have major toxicological consequences for mammals, it is to be expected that CGA 321113 would be less toxic than the parent molecule. CGA 321113 was found to be 10-30 times less hepatotoxic (NOEC 100 µg/mL) than trifloxystrobin (NOEC 3 µg/mL) in rat hepatocytes in-vitro. Furthermore, CGA 321113 is contributing to > 10 % (in bile of male and female rats) of absorbed dose of trifloxystrobin in the rat ADME study. Therefore, CGA 321113 is considered as characterized by the toxicity studies performed with parent compound. The ADI of trifloxystrobin (0.1 mg/kg bw/day) is adequate for the consumer risk assessment.
CGA 321113 is not considered relevant and is further evaluated in Step 4.

10.2.4 STEP 4: Exposure assessment – threshold of concern approach

CGA 321113 was not considered relevant in the hazard assessment of Step 3.

The highest PEC_{gw} for metabolite **CGA 321113** exceed the threshold of concern limit of 0.75 µg/L (but <10 µg/L). A further assessment in Step 5 is required.

10.2.5 STEP 5: Refined risk assessment

Metabolite **CGA 321113** has a PEC_{gw} between 0.75 µg/L and 10 µg/L. A refined risk assessment of the potential consumer exposure is presented here.

CGA 321113: The consumer risk assessment demonstrates an acceptable risk. The estimated safety margin including potential exposure via drinking water for CGA 321113 are 0.306 % of ADI (infant), 0.204 % ~~0.17%~~ of ADI (child), 0.068 % ~~0.058%~~ of ADI (adult).

Justification for the selected ADI:
The ADI for trifloxystrobin is 0.1 mg/kg bw/d based on a 2-year rat study and an uncertainty factor of 100 as stated in EFSA Journal 2017;15(10):4989.
Metabolism and toxicity data as well as structural considerations indicate that the metabolite CGA 321113 is likely to be less toxic than trifloxystrobin. It is therefore concluded that, as a worst case assumption, the ADI of the parent compound (0.1 mg/kg bw/day) is adequate for the consumer risk assessment.

Calculation of risk (% ADI) for 5-kg bottle-fed infant (consuming 0.75 L/day):

Table 10.2-2: Intake of CGA 321113 via drinking water

Consumer, body weight and water consumption	Max. groundwater concentration [µg/L]	Intake [µg/person]	Intake [µg/kg bw/d]	Usage of ADI [%]
5 kg bottle-fed infant, 0.75 L/day	2.042	1.532	0.306	0.306

Calculation of risk (% ADI) for 10 ~~12~~-kg child (consuming 1.0 L/day):

Table 10.2-3: Intake of CGA 321113 via drinking water

Consumer, body weight and water consumption	Max. groundwater concentration [µg/L]	Intake [µg/person]	Intake [µg/kg bw/d]	Usage of ADI [%]
10 ~~12~~-kg child, 1.0 L/day	2.042	2.042	0.204 ~~0.170~~	0.204 ~~0.17%~~

Calculation of risk (% ADI) for 60 ~~70~~-kg adult (consuming 2.0 L/day):

Table 10.2-4: Intake of CGA 321113 via drinking water

Consumer, body weight and water consumption	Max. groundwater concentration [µg/L]	Intake [µg/person]	Intake [µg/kg bw/d]	Usage of ADI [%]
60 ~~70~~ kg adult, 2 L/day	2.042	4.084	0.068 ~~0.058~~	0.068 ~~0.058~~

Risk assessment: Combined consumer exposure via plant commodities and drinking water

In order to evaluate the potential total dietary exposure to CGA 321113 in addition potential residues resulting from commodities of plant and animal origin need to be considered.

For reason of simplification, the worst case assumption is made that plant residues consist to 100% of CGA 321113. Under actual agronomic conditions, residue levels of CGA 321113 are expected to be considerably less compared to the parent compound.

For the chronic consumer exposure assessment either MRLs or median residue values multiplied by a conversion factor are used. The conversion factors – as suggested by EFSA - take into account additional residues of CGA 321113 that might be present in certain crops.

Although in case of the MRLs, the metabolite CGA 321113 is not included, the calculation using the MRL represents a worst case, since the MRL values are generally higher than the median residue of trifloxystrobin multiplied by the conversion factor to accommodate for residues of metabolite CGA 321113.

The input values used for the chronic dietary exposure calculation are summarised in Section B7.

The calculated exposure was then compared with the toxicological reference value derived for trifloxystrobin (ADI: 0.1 mg/kg bw/d). The results of the intake calculation are presented in Section B7.

No long-term consumer intake concerns were identified for any of the European diets incorporated in the EFSA PRIMo. The total calculated intake values accounted up to 50% of the ADI (NL toddler).

The combined consumer exposure via plant and animal commodities and drinking water accounts to 50% (rounded) of the ADI (drinking water only accounting to max. 0.306% of the ADI).

10.3 Relevance assessment of metabolite NOA 413161

The relevance of the groundwater metabolite NOA 413161 has already been assessed and the assessment agreed at EU level (see EFSA Journal 2017;15(10):4989), but the relevance assessment is not applicable 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 not valid with regard to the PECgw calculated for the GAP and groundwater scenarios considered in this dRR). Therefore, the assessment and conclusions are presented here.

Trifloxystrobin metabolite **NOA 413161** 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 below.

Table 10.3-1: Summary of the relevance assessment for metabolite NOA 413161

	Assessment step		Result of assessment	
	STEP 1		Metabolite of no concern?	No
Quantification of groundwater contamination	STEP 2		Max PEC_{gw}	7.278 µg/L
			Based on	FOCUS PEARL for scenario Jokioinen, Lambs lettuce, lettuce, rocket salad (modelling crop: Cabbage II (early)), 2 × 200 g a.s./ha
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 oral toxicity to rats (LD_{50} > 2000 mg/kg bw), 28-day oral rat: NOAEL = 150 mg/kg bw per day based on changes in urine parameters. Comparative in vitro experiment: NOA 413161 caused significantly less inhibition of mitochondrial respiration than trifloxystrobin.
			Classification of parent	Skin Sens. 1, H317 Effects on or via lactation, H362
			Classification of metabolite	Not classified
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)
	STEP 5		Refined risk assessment	Acceptable
			Predicted exposure (% of ADI)	Intake by means of drinking water: 0.728 (infant, 5 kg) % ADI 0.485 (child, 10 ~~12~~ kg) ~~0.404~~% ADI 0.162 (adult, 60 ~~70~~ kg) ~~0.130~~% ADI
			ADI based on	0.15 mg/kg bw/d (ADI for NOA 413161 according to EFSA Journal 2017;15(10):4989)

10.3.1 STEP 1: Exclusion of degradation products of no concern

The metabolite does not meet the criteria for products of no concern as defined in step 1 of the guidance and therefore they need further assessment.

It cannot be excluded as a product of no concern as they are not:

- CO2 or an inorganic compound, not containing a heavy metal;
- an organic compound of aliphatic structure, with a chain length of 4 or less, consisting 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;
- 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.

10.3.2 STEP 2: Quantification of potential groundwater contamination

PEC_{gw} calculations after leaching from soil were performed (Part B, Section 8 for details data). The uses for which metabolite concentrations were considered to exceed 0.1 µg/L are listed in the table above. **Therefore, further assessment of the relevance of the metabolite is required** in terms of biological, genotoxicological and toxicological activity according to EU Guidance Document SANCO/221/2000, rev. 10 (2003).

10.3.3 STEP 3: Hazard assessment – identification of relevant metabolites

10.3.3.1 STEP 3, Stage 1: screening for biological activity

Tests was conducted to investigate the biological activity of the lysimeter metabolite NOA 413161. A short summary of the results given in Annex II, point 3 of trifloxystrobin (M-069325-01-1) [2] is presented in the following paragraph:

"It could be shown that both mono- and both bis-acids have lost most of their biological activity compared to the active substance CGA 279202. In two separate in-vivo tests it could be shown that the acid metabolite of CGA 279202, CGA 321113 is by a factor of thousand less biological active than the parent. From the four metabolites tested, CGA 321113 showed the highest biological activity. CGA 373466 (ZE-isomer of CGA 321113) as well as the bis-acids are again a factor of ten less active than the monoacid CGA 321113. The effect that the EE isomer is biological more active than the ZE could also be shown for the parent compound in in-vivo tests, thereby the biological activity of CGA 279202 and its isomers decreased in the following order: EE > EZ ≥ ZE (ZZ was not active). In these in-vivo tests no biological activity was detected for the metabolite CGA 321113."

The Guidance Document states that metabolites with a biological activity comparable or higher than the parent are considered as relevant. This metabolite is considered not relevant and is further evaluated in Step 3, stage 2.

10.3.3.2 STEP 3, Stage 2: screening for genotoxicity

NOA 413161 was screened for genotoxic activity by the following data package of *in vitro* genotoxicity studies: Ames test, gene mutation test with mammalian cells, and a chromosome aberration test. NOA 413161 was non-genotoxic as shown by a negative Ames test, negative gene mutation test with mammalian cells, negative chromosome aberration test.

Thus, the metabolite is considered to be 'non-relevant' regarding genotoxicity and is further evaluated in Step 3.

10.3.3.3 STEP 3, Stage 3: screening for toxicity

The parent compound, trifloxystrobin, is classified for health effects in accordance with CLP Regulation Annex VI, Part 3 List of harmonized classifications (EC-Regulation 790/2009) only for skin sensitization, Category 1 (H317 May cause an allergic skin reaction). In addition, following the ECHA/RAC 50 meeting for trifloxystrobin, the Committee agreed to classify trifloxystrobin for effects through or via lactation (may cause harm to breast-fed children, Lact.; H362).

The parent, trifloxystrobin, to **NOA 413161** is not classified as acutely or chronically toxic or very toxic / for reproductive toxicity / as a carcinogen in category 1 or 2 (or corresponding classification in accordance to CLP 1272/2008). There are no reasons to expect that NOA 413161 may be toxic or highly toxic.

NOA 413161 is non-toxic (LD_{50} > 2000 mg/kg bw) after acute oral exposure, in a 28-day oral rat study a NOAEL was established at 150 mg/kg bw per day based on changes in urine parameters in males at 1000 mg/kg bw per day, in a comparative in vitro experiment NOA 413161 caused significantly (900-1000 times) less inhibition of mitochondrial respiration than trifloxystrobin. Since significant inhibition of cellular

[2] Ohs, P., Tier II, IIA, 3: Data on application, Report No.: MO-02-008756, Edition Number: M-069325-01-1, 2002. Can be made available on request.

respiration is likely to have major toxicological consequences for mammals, it is to be expected that NOA 413161 would be less toxic than the parent molecule. NOA 413161 was found to be clearly less hepatotoxic (NOEC >1000 µg/mL) than trifloxystrobin (NOEC 3 µg/mL) in rat hepatocytes in-vitro. The ADI for NOA 413161 is 0.15 mg/kg bw/d based on a 28-day study with rats and an uncertainty factor of 1000 as stated in EFSA Journal 2017;15(10):4989.

NOA 413161 is not considered relevant and is further evaluated in Step 4.

10.3.4 STEP 4: Exposure assessment – threshold of concern approach

NOA 413161 was not considered relevant in the hazard assessment of Step 3.

The highest PEC_{gw} for metabolite **NOA 413161** exceed the threshold of concern limit of 0.75 µg/L (but <10 µg/L). A further assessment in Step 5 is required.

10.3.5 STEP 5: Refined risk assessment

Metabolite **NOA 413161** has a PEC_{gw} between 0.75 µg/L and 10 µg/L. A refined risk assessment of the potential consumer exposure is presented here.

NOA 413161: The consumer risk assessment demonstrates an acceptable risk. The estimated safety margin including potential exposure via drinking water for NOA 413161 are 0.728 % of ADI (infant), 0.485 ~~0.404~~ % of ADI (child), 0.162 ~~0.138~~% of ADI (adult).

Justification for the selected ADI:
The ADI for NOA 413161 is 0.15 mg/kg bw/d based on a 28-day study with rats and an uncertainty factor of 1000 as stated in EFSA Journal 2017;15(10):4989.

Calculation of risk (% ADI) for 5-kg bottle-fed infant (consuming 0.75 L/day):

Table 10.3-2: Intake of NOA 413161 via drinking water

Consumer, body weight and water consumption	Max. groundwater concentration [µg/L]	Intake [µg/person]	Intake [µg/kg bw/d]	Usage of ADI [%]
5 kg bottle-fed infant, 0.75 L/day	7.278	5.459	1.092	0.728

Calculation of risk (% ADI) for 10 ~~12~~-kg child (consuming 1.0 L/day):

Table 10.3-3: Intake of NOA 413161 via drinking water

Consumer, body weight and water consumption	Max. groundwater concentration [µg/L]	Intake [µg/person]	Intake [µg/kg bw/d]	Usage of ADI [%]
10 ~~12~~ kg child, 1.0 L/day	7.278	7.278	0.728 ~~0.606~~	0.485 ~~0.404~~

Calculation of risk (% ADI) for 60 ~~70~~-kg adult (consuming 2.0 L/day):

Table 10.3-4: Intake of NOA 413161 via drinking water

Consumer, body weight and water consumption	Max. groundwater concentration [µg/L]	Intake [µg/person]	Intake [µg/kg bw/d]	Usage of ADI [%]
60 ~~70~~ kg adult, 2 L/day	7.278	14.556	0.243 ~~0.207~~	0.162 ~~0.138~~

Risk assessment: Combined consumer exposure via plant commodities and drinking water
NOA 413161 was not present at levels above 0.01 mg/kg in human food plant commodities as seen in the plant metabolism studies. The metabolite was only present at levels above 0.01 mg/kg in the feeding item cereal straw (up to 2%TRR and 0.11 mg/kg). The metabolite was not determined in livestock metabolism studies.
Transfer of the straw metabolite into food of animal origin at measurable amounts is unlikely. Exposure of consumers to the metabolite NOA 413161 by intake of food of animal origin is not expected.

10.4 Relevance assessment of metabolite NOA 413163

The relevance of the groundwater metabolite NOA 413163 has already been assessed and the assessment agreed at EU level (see EFSA Journal 2017;15(10):4989), but the relevance assessment is not applicable 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 not valid with regard to the PEC_{gw} calculated for the GAP and groundwater scenarios considered in this dRR). Therefore, the assessment and conclusions are presented here.

Trifloxystrobin metabolite **NOA 413163** 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 below.

Table 10.4-1: Summary of the relevance assessment for metabolite NOA 413163

	Assessment step		**Result of assessment**	
	STEP 1		Metabolite of no concern?	No
Quantification of groundwater contamination	STEP 2		Max PEC_{gw}	9.894 µg/L
			Based on	FOCUS PEARL for scenario Jokioinen, Apples II (early), 2 × 200 g a.s./ha
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 oral toxicity to rats (LD_{50} > 2000 mg/kg bw), Comparative in vitro experiment: NOA 413163 caused significantly less inhibition of mitochondrial respiration than trifloxystrobin. 28-day oral rat: NOAEL > 1000 mg/kg bw per day conducted with the mixture of NOA 413161 / NOA 413163.
			Classification of parent	Skin Sens. 1, H317 Effects on or via lactation, H362
			Classification of metabolite	Not classified
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)
	STEP 5		Refined risk assessment	Acceptable
			Predicted exposure (% of ADI)	Intake by means of drinking water: 0.285 (infant, 5 kg) 0.190 (child, 10 ~~12~~ kg) ~~0.158~~ % ADI 0.063 (adult, 60 ~~70~~ kg) ~~0.054~~ % ADI

		ADI based on	0.52 mg/kg bw/d (ADI for NOA 413163 according to EFSA Journal 2017;15(10):4989)

10.4.1 STEP 1: Exclusion of degradation products of no concern

The metabolite does not meet the criteria for products of no concern as defined in step 1 of the guidance and therefore they need further assessment.

It cannot be excluded as a product of no concern as they are not:

- CO2 or an inorganic compound, not containing a heavy metal;
- an organic compound of aliphatic structure, with a chain length of 4 or less, consisting 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;
- 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.

10.4.2 STEP 2: Quantification of potential groundwater contamination

PEC_{gw} calculations after leaching from soil were performed (Part B, Section 8 for details data). The uses for which metabolite concentrations were considered to exceed 0.1 µg/L are listed in the table above. **Therefore further assessment of the relevance of the metabolite is required** in terms of biological, genotoxicological and toxicological activity according to EU Guidance Document SANCO/221/2000, rev. 10 (2003).

10.4.3 STEP 3: Hazard assessment – identification of relevant metabolites

10.4.3.1 STEP 3, Stage 1: screening for biological activity

Tests was conducted to investigate the biological activity of the lysimeter metabolite CGA°413163. A short summary of the results given in Annex II, point 3 of trifloxystrobin (M-069325-01-1) [3] is presented in the following paragraph:

"It could be shown that both mono- and both bis-acids have lost most of their biological activity compared to the active substance CGA 279202. In two separate in-vivo tests it could be shown that the acid metabolite of CGA 279202, CGA 321113 is by a factor of thousand less biological active than the parent. From the four metabolites tested, CGA 321113 showed the highest biological activity. CGA 373466 (ZE-isomer of CGA 321113) as well as the bis-acids are again a factor of ten less active than the monoacid CGA 321113. The effect that the EE isomer is biological more active than the ZE could also be shown for the parent compound in in-vivo tests, thereby the biological activity of CGA 279202 and its isomers decreased in the following order: EE > EZ ≥ ZE (ZZ was not active). In these in-vivo tests no biological activity was detected for the metabolite CGA 321113."

The Guidance Document states that metabolites with a biological activity comparable or higher than the parent are considered as relevant. This metabolite is considered not relevant and is further evaluated in Step 3, stage 2.

[3] Ohs, P., Tier II, IIA, 3: Data on application, Report No.: MO-02-008756, Edition Number: M-069325-01-1, 2002. Can be made available on request.

10.4.3.2 STEP 3, Stage 2: screening for genotoxicity

NOA 413163 was screened for genotoxic activity by the following data package of *in vitro* genotoxicity studies: Ames test (with NOA 413163) and gene mutation test with mammalian cells, and a chromosome aberration test (both with a mixture of NOA 413161/NOA 413163). NOA 413163 was non-genotoxic as shown by a negative Ames test, negative gene mutation test with mammalian cells, and negative chromosome aberration test.

Thus, the metabolite is considered to be 'non-relevant' regarding genotoxicity and is further evaluated in Step 3.

10.4.3.3 STEP 3, Stage 3: screening for toxicity

The parent compound, trifloxystrobin, is classified for health effects in accordance with CLP Regulation Annex VI, Part 3 List of harmonized classifications (EC-Regulation 790/2009) only for skin sensitization, Category 1 (H317 May cause an allergic skin reaction). In addition, following the ECHA/RAC 50 meeting for trifloxystrobin, the Committee agreed to classify trifloxystrobin for effects through or via lactation (may cause harm to breast-fed children, Lact.; H362).

The parent, trifloxystrobin, to **NOA 413163** is not classified as acutely or chronically toxic or very toxic / for reproductive toxicity / as a carcinogen in category 1 or 2 (or corresponding classification in accordance to CLP 1272/2008). There are no reasons to expect that NOA 413163 may be toxic or highly toxic.

NOA 413163 is non-toxic (LD50 > 2000 mg/kg bw) after acute oral exposure, in a 28-day oral rat study a NOAEL was established at 1000 mg/kg bw per day conducted with the mixture of NOA 413161 / NOA 413163, in a comparative in vitro experiment NOA 413163 caused significantly (900-1000 times) less inhibition of mitochondrial respiration than trifloxystrobin. Since significant inhibition of cellular respiration is likely to have major toxicological consequences for mammals, it is to be expected that NOA 413163 would be less toxic than the parent molecule. NOA 413163 was found to be less (NOEC >1000 µg/mL) hepatotoxic than trifloxystrobin (NOEC 3 µg/mL) in rat hepatocytes in-vitro. The ADI for NOA 413163 is 0.52 mg/kg bw/d based on the 28-day study with rats with the mixture of NOA 413161 and NOA 413163 and corrected for the content of the metabolite in the mixture and an uncertainty factor of 1000 (EFSA Journal 2017;15(10):4989).

NOA 413163 is not considered relevant and is further evaluated in Step 4.

10.4.4 STEP 4: Exposure assessment – threshold of concern approach

NOA 413163 was not considered relevant in the hazard assessment of Step 3.

The highest PEC_{gw} for metabolite **NOA 413163** exceed the threshold of concern limit of 0.75 µg/L (but <10 µg/L). A further assessment in Step 5 is required.

10.4.5 STEP 5: Refined risk assessment

Metabolite **NOA 413163** has a PEC_{gw} between 0.75 µg/L and 10 µg/L. A refined risk assessment of the potential consumer exposure is presented here.

NOA 413163: The consumer risk assessment demonstrates an acceptable risk. The estimated safety margin including potential exposure via drinking water for NOA 413163 are 0.285 % of ADI (infant), 0.190 ~~0.158~~% of ADI (child), 0.063 ~~0.054~~% of ADI (adult).

Justification for the selected ADI:
The ADI for NOA 413163 is 0.52 mg/kg bw/d based on a 28-day study with rats with the mixture of NOA 413161 and NOA 413163 and corrected for the content of the metabolite in the mixture and an uncertainty

factor of 1000 as stated in EFSA Journal 2017;15(10):4989.

Calculation of risk (% ADI) for 5-kg bottle-fed infant (consuming 0.75 L/day):

Table 10.4-2: Intake of NOA 413163 via drinking water

Consumer, body weight and water consumption	Max. groundwater concentration [µg/L]	Intake [µg/person]	Intake [µg/kg bw/d]	Usage of ADI [%]
5 kg bottle-fed infant, 0.75 L/day	9.894	7.421	1.484	0.285

Calculation of risk (% ADI) for 10-kg child (consuming 1.0 L/day):

Table 10.4-3: Intake of NOA 413163 via drinking water

Consumer, body weight and water consumption	Max. groundwater concentration [µg/L]	Intake [µg/person]	Intake [µg/kg bw/d]	Usage of ADI [%]
10 ~~12~~ kg child, 1.0 L/day	9.894	9.894	0.989 ~~0.824~~	0.190 ~~0.158~~

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

Table 10.4-4: Intake of NOA 413163 via drinking water

Consumer, body weight and water consumption	Max. groundwater concentration [µg/L]	Intake [µg/person]	Intake [µg/kg bw/d]	Usage of ADI [%]
60 ~~70~~ kg adult, 2 L/day	9.894	19.788	0.330 ~~0.282~~	0.063 ~~0.054~~

Risk assessment: Combined consumer exposure via plant commodities and drinking water

NOA 413163 was not present at levels above 0.01 mg/kg in human food plant commodities as seen in the plant metabolism studies. The metabolite was only present at levels above 0.01 mg/kg in the feeding item cereal hay and straw (up to 6%TRR and 0.35 mg/kg). The metabolite was not determined in livestock metabolism studies.

Transfer of the straw metabolite into food of animal origin at measurable amounts is unlikely. Exposure of consumers to the metabolite NOA 413163 by intake of food of animal origin is not expected.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

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

Please note that all data mentioned as part of DAR, RAR, or EFSA journals are considered as relied on.

Trifloxystrobin

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study** **Y/N**	Owner
KCA 5.8.1 /09	xxxx	1998	NOA 413163 tech. (metabolite of CGA 279202) - Acute oral toxicity in the rat (limit test) xxx, Report No.: 983103, Edition Number: M-052684-01-1 Date: 1998-08-18 GLP/GEP: yes, unpublished	Y	Bayer
KCA 5.8.1 /10	Deparade, E.	1998	NOA 413163 tech. (metabolite of CGA 279202) - Salmonella and escherichia/mammalian-microsome mutagenicity test Novartis Crop Protection AG, Basel, Switzerland Bayer CropScience, Report No.: 983104, Edition Number: M-052705-01-1 Date: 1998-09-29 GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /11	Herbold, B.	2002	CGA 279202-NOA 413161/413163 - In vitro chromosome aberration test with Chinese hamster V79 cells Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 32151, Edition Number: M-069747-01-1 Date: 2002-07-02 GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /12	Herbold, B.	2002	CGA 279202-NOA 413161/413163 - V79/HPRT-test in vitro for the detection of induced forward mutations Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 32150, Edition Number: M-069760-01-1 Date: 2002-07-02 GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /13	xxx	1998	NOA 413161 tech. (metabolite of CGA 279202) - Acute oral toxicity in the rat (limit test) xxx, Report No.: 983068, Edition Number: **M-052694-01-1** Date: 1998-08-18 GLP/GEP: yes, unpublished	Y	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study** **Y/N**	**Owner**
KCA 5.8.1 /14	Deparade, E.	1998	NOA 413161 tech. (metabolite of CGA 279202) - Salmonella and escherischia/mammalian-microsome mutagenicity test Novartis Crop Protection AG, Basel, Switzerland Bayer CropScience, Report No.: 983069, Edition Number: M-054210-01-1 Date: 1998-09-16 GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /15	Ogorek, B.	1999	NOA 413161 (metabolite of CGA 279202) - Cytogenetic test on chinese hamster cells in vitro Novartis Crop Protection AG, Basel, Switzerland Bayer CropScience, Report No.: 993094, Edition Number: M-054214-01-1 Date: 1999-12-13 GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /16	Ogorek, B.	2000	NOA 413161 (metabolite of CGA 279202) - Gene mutation test with chinese hamster cells V79 Novartis Crop Protection AG, Basel, Switzerland Bayer CropScience, Report No.: 993095, Edition Number: M-054225-01-1 Date: 2000-04-25 GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /17 KCA 5.3.1 /03	xxx	2000	NOA 413161 tech. (metabolite of CGA 279202 tech.) - 28-day subacute oral toxicity study in rats xxx, Report No.: 993090, Edition Number: M-137124-01-1 Date: 2000-03-30 GLP/GEP: yes, unpublished	Y	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study** **Y/N**	**Owner**
KCA 5.8.1 /18	Heimann, K. G.	2001	CGA 321113 - Statement on the relevance of the Trifloxystrobin metabolite Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: MO-01-000794, Edition Number: M-031965-01-1 Date: 2001-01-18 GLP/GEP: no, unpublished	N	Bayer
KCA 5.8.1 /19	Bouis, P.	1997	CGA 279202 and CGA 321113 - Cytotoxicity in primary cultured rat hepatocytes and effects on mitochondrial function of rat liver Novartis Crop Protection AG, Basel, Switzerland Bayer CropScience, Report No.: CB97/59, Edition Number: M-039240-01-1 EPA MRID No.: 44496720 Date: 1997-12-17 GLP/GEP: no, unpublished	N	Bayer
KCA 5.8.1 /20	Freyberger, A.	2002	Effects of trifloxystrobin (CGA 279202) and its metabolites CGA 321113, CGA 373466, NOA 413161 and NOA 413163 on succinate-supported rat liver mitochondrial respiration Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 31746, Edition Number: M-034840-02-1 Date: 2002-02-06 **...Amended: 2003-03-03** GLP/GEP: no, unpublished	N	Bayer
KCA 5.8.1 /21	Wasinska-Kempka, G.	2002	Investigation of the hepatotoxic potential of trifloxystrobin and its metabolites on primary rat hepatocytes in an in vitro model Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 31822, Edition Number: M-090653-02-1 Date: 2002-01-09 **...Amended: 2002-03-01** GLP/GEP: no, unpublished	N	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study** **Y/N**	Owner
KCA 5.8.1 /30	Sokolowski, A.	2011	Salmonella typhimurium reverse mutation assay with CGA 279202-CGA 321113 Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1390501, Edition Number: M-406346-01-1 Date: 2011-04-27 GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /31	Wollny, H. E.	2011	CGA 279202-CGA 321113 - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT) Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1390503, Edition Number: M-411413-01-1 Date: 2011-07-27 GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /32	Hall, C.	2011	CGA 279202-CGA 321113 - In vitro chromosome aberration test in Chinese hamster V79 cells Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1390502, Edition Number: M-413745-01-1 Date: 2011-09-08 GLP/GEP: yes, unpublished	N	Bayer
KCA 5.8.1 /33	xxx	2013	CGA 279202-CGA 321113: Micronucleus test in bone marrow cells of the mouse xxx, Report No.: 1578200, Edition Number: M-463614-01-1 Date: 2013-01-01 GLP/GEP: yes, unpublished	Y	Bayer
KCA 5.8.1 /34	xxx	2013	In vivo unscheduled DNA synthesis in rat hepatocytes with trifloxystrobin-CGA 321113 xxx, Report No.: 1504401, Edition Number: M-458428-01-1 Date: 2013-06-17 GLP/GEP: yes, unpublished	Y	Bayer

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 5.8.1 /35	xxx	2003	CGA 279202-CGA 373466 - Study for subacute oral toxicity in rats (feeding study for 4 weeks and 4 weeks recovery period) xxx, Report No.: AT00343, Edition Number: M-088404-01-1 Date: 2003-03-31 GLP/GEP: yes, unpublished	Y	Bayer
KCA 5.8.1 /36	xxx	2003	CGA 279202-NOA 413161/413163 - Study for subacute oral toxicity in rats (4-week application by gavage and 4 weeks recovery period) xxx, Report No.: AT00342, Edition Number: M-084123-01-1 Date: 2003-03-31 GLP/GEP: yes, unpublished	Y	Bayer

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	Vertebrate study 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	Vertebrate study Y/N	Owner
-	-	-	-	-	-