FINAL REGISTRATION REPORT

# Part B

# Section 10

# Assessment of the relevance of metabolites in groundwater

Detailed summary of the risk assessment

Product code: CHR/H/TERIZ 650 WG

Product name(s): Undito 650 WG, Jotamun 650 WG, Metodus 650 WG

Chemical active substance(s):

Terbuthylazine, 400 g/kg

Isoxaflutole, 100 g/kg

Mesotrione, 150 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT – renewal of authorisation (Poland)

Applicant: Innvigo Sp. z o.o.

Submission date: October 2019

MS Finalisation date: December 2021; June 2023

# Version history

| When | What |
|---|---|
| October 2019 | New data for isoxaflutole based on the renewal of active substance. New data marked in yellow |
| December 2021 | New data assessed by zRMS. |
| June 2023 | Final Registration Report |
| | |

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# 10 Relevance of metabolites in groundwater

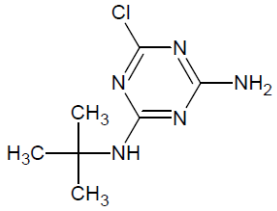
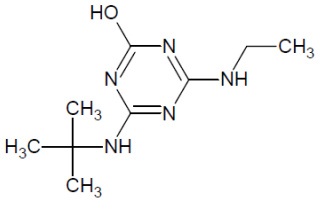
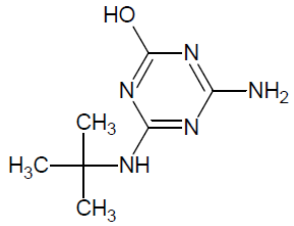
| **Evaluator's comments:** |
|---|
| The detailed metabolite assessment of terbuthylazine and mesotrione are presented in the previous dRR provided in 2017 and finalized in 2019. |

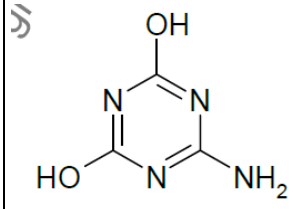
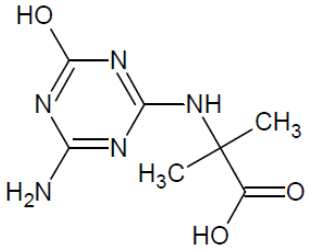
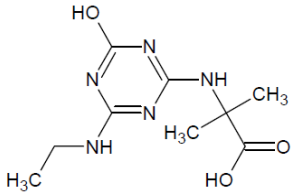
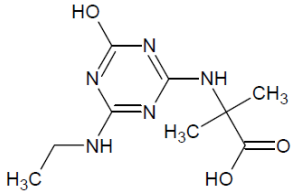
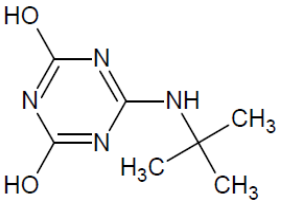
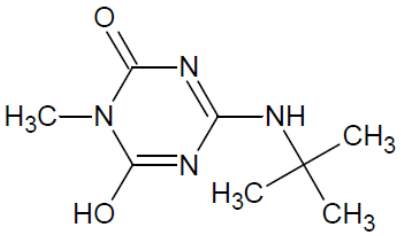
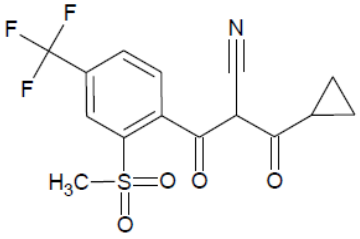
## 10.1 General information

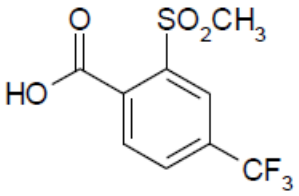
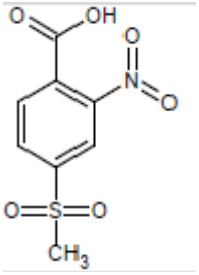
The metabolites MT1, MT13, MT14, LM1, LM2, LM3, LM4,LM5,LM6 (terbuthylazine metabolites); RPA 202248, RPA 203328 ( isoxaflutole metabolites); MNBA (mesotrione metabolites) are predicted to occur in groundwater at concentrations above 0.1 µg/L (see PART B Section 8 of CHR/H/TERIZ dRR). Assessment of the 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 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 cGAP evaluation in chapter KCP 9.2.4 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 assessmnent | |
|---|---|---|---|---|
| Terbuthylazine | MT1<br>desethyl-terbuthylazine<br>(GS 26379) |  | Max $PEC_{gw}$<br><br>Based on: | 0.59 µg/L<br><br>Focus PEARL 4.4.4<br>Hamburg |
| | MT13<br>Hydroxy-terbuthylazine<br>Or 2-hydroxy terbuthylazine<br>GS 23158 |  | Max $PEC_{gw}$<br><br>Based on: | 14.95 µg/L<br><br>Focus PEARL 4.4.4<br>Hamburg |
| | MT14<br>desethyl-hydroxyterbuthylazine<br>or<br>desethyl-2-hydroxy terbuthylazine<br>GS 28620 |  | Max $PEC_{gw}$<br><br>Based on: | 3.45 µg/L<br><br>Focus PEARL 4.4.4<br>Hamburg |

| Name of active substance | Metabolite name and code | Structural/molecular formula | Trigger for relevance assessmnent | |
|---|---|---|---|---|
| | LM1<br>MT24 |  | Based on, lysimeter studies: | 0.15µg/l |
| | LM 2<br>MT28 |  | Based on lysimeter: | 0.10 µg/l |
| | LM 3<br>SM9<br>CSCD692760<br>SYN546009 |  | Based on lysimeter: | Notifiers simulations gave PECgw values in the range from 0.448 to 2.21 µg/l. |
| | LM4<br>SM4<br>CSAA404949<br>GS40436 |  | Max $PEC_{gw}$<br><br>Based on LM3: | Notifiers simulations gave PECgw values in the range from 0.448 to 2.21 µg/l. |
| | LM5<br>MT23<br>SM12<br>GS 16984 |  | Max $PEC_{gw}$<br><br>Based on: | Notifiers simulations gave PECgw values in the range from 0.157 to 2.08 µg/l |
| | LM6<br>SM6<br>CSCD648241<br>SYN545666 |  | Max $PEC_{gw}$<br><br>Based on: | Notifiers simulations gave PECgw values in the range from 0.296 to 1.91 µg/l |
| Isoxaflutole | RPA 202248 |  | Max $PEC_{gw}$<br><br>Based on: | 1.15 µg/L (appl. rate: 100g/ha, once each year)<br><0.1 µg/L (appl. rate: 80 g/ha, once every three |

| Name of active substance | Metabolite name and code | Structural/molecular formula | Trigger for relevance assessmnent | |
|---|---|---|---|---|
| | | | | years)<br><br>Focus PEARL 4.4.4 Hamburg |
| | RPA 203328 |  | Max $PEC_{gw}$<br><br>Based on: | 3.87 µg/L<br><br>Focus PEARL 4.4.4 Hamburg |
| Mesotrione | MNBA |  | Max $PEC_{gw}$<br><br>Based on: | 0.2 µg/L<br><br>Focus PEARL 4.4.4 Hamburg |
| Mesotrione | AMBA | | Max PECgw<br><br>Based on: | 0.16 µg/L<br><br>FOCUS PEARL 4.4.4 |

## 10.2 Relevance assessment of MT1

**Summary:**

The relevance of the groundwater metabolite MT1 has already been assessed and the assessment agreed at EU level, 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 ). MT1 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 MT1 according to the Addendum confirmatory data 2015.**

| | Assessment step | | Result of assessment | |
|---|---|---|---|---|
| | STEP 1 | | Metabolite of no concern? | Yes |
| tion of groundwater | STEP 2 | | Max $PEC_{gw}$ | 0.59 µg/L |
| | | | Based on | FOCUS PEARL; Hamburg |
| Hazard assess | STEP 3 | Stage 1 | Biological activity comparable to the parent? | Yes |

| | | | | |
|---|---|---|---|---|
| | | Stage 2 | Genotoxic properties of metabolite | Non-genotoxic |
| | | Stage 3 | Toxic properties of metabolite; | Not toxic or very toxic ( T or T+) |
| | | | Classification of parent | not currently classified as toxic or very toxic |
| | | | Classification of metabolite | not currently classified as toxic or very toxic |
| **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 | Not required |
| | | | Predicted exposure (% of ADI) | Not required |
| | | | ADI based on | N/A |

* N/A: not applicable

### 10.2.1 STEP 1: Exclusion of degradation products of no concern

Could not be excluded

### 10.2.2 STEP 2: Quantification of potential groundwater contamination

| | |
|---|---|
| Max $PEC_{gw}$ | 0.59 µg/L |
| Based on: | Focus PEARL 4.4.4 Hamburg |

In the field leaching study in Northern Italy annual average concentrations ranged from <0.01 up to 0.73µg/l in fields receiving basin irrigation. The maximum annual average concentration in fields receiving more conventional irrigation was 0.22µg/l. The conditions during the field leaching study in Northern Italy are likely to represent highly vulnerable conditions in terms of groundwater contamination in the EU due to the combination of soils, climate and extensive use of terbuthylazine on maize in the areas investigated. In addition this metabolite was not detected in an extensive and targeted German groundwater monitoring program. In further groundwater monitoring studies in Italy, Spain and Portugal the 90th percentile concentration was always <0.1µg/l. On the basis of the additional information from field leaching and groundwater monitoring programs it is clear that the first tier FOCUS groundwater exposure assessment represents a conservative assessment and such high concentrations are unlikely to be encountered under realistic use conditions**.**

### 10.2.3 STEP 3: Hazard assessment – identification of relevant metabolites

#### 10.2.3.1 STEP 3, Stage 1: screening for biological activity

Data on MT1 (desethyl-terbuthylazine) which showed some signs of herbicidal activity. In addition, screening data (Corbin J, 2009) was provided as part of the resubmission and is presented in this Additional Report (see Section B.9.9.2. See also Attachment 1 DAR 2010). The conclusion was that the metabolite MT1 is herbicidally active. The biological activity of the metabolite is

broadly similar to that of terbuthylazine when applied at a dose at which the parent demonstrates good herbicidal activity on key species (common amaranth, fat hen, common chickweed, and wild oats) at the field rate of 750 g a.s./ha. On this basis this metabolite should be considered as being 'relevant' in terms of the guidance document.

### 10.2.3.2 STEP 3, Stage 2: screening for genotoxicity

Although weakly mutagenic in vitro (gene mutation) MT1 was negative in two in vivo assays and can be regarded as non-genotoxic.

### 10.2.3.3 STEP 3, Stage 3: screening for toxicity

MT1 was found to be of comparatively high acute oral toxicity in the rat (LD50 =236 mg/kg bw. Based on a comparison with the 90 day study with MT1 and the two 90 day studies with terbuthylazine in the original DAR it appears that MT1 produces some but not all the effects seen in the terbuthylazine studies at similar dose levels. It appears to have similar or slightly lower short term toxicity than parent. The 90 day study is not considered suitable for determining a reference value for MT1 (no NOAEL and lacking detail)

### 10.2.4 STEP 4: Exposure assessment – threshold of concern approach

PECgw value is below TTC 0.75 µg/L, therefore STEP 5 of refined risk assessment is not necessary.

### 10.2.5 STEP 5: Refined risk assessment

**Not applicable-please refer to point 10.2**

## 10.3 Relevance assessment of MT 13

**Summary:**

The relevance of the groundwater metabolite MT13 has already been assessed and the assessment agreed at EU level (see DAR 2010) , 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 ). MT13 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 10.3-1: Summary of the relevance assessment for MT13 according to the DAR Additional report 2010.**

| | **Assessment step** | **Result of assessment** | |
|---|---|---|---|
| | STEP 1 | Metabolite of no concern? | Yes |
| **groundwa** | STEP 2 | Max $PEC_{gw}$ | 14.95 µg/L |

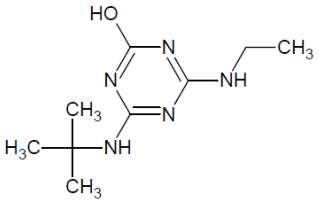
| | | | | |
|---|---|---|---|---|
| | | | Based on | FOCUS PEARL, Hmburg |
| **Hazard assessment** | STEP 3 | Stage 1 | Biological activity comparable to the parent? | no |
| | | Stage 2 | Genotoxic properties of metabolite | Non-genotoxic |
| | | Stage 3 | Toxic properties of metabolite; | Not toxic or very toxic ( T or T+) |
| | | | Classification of parent | not currently classified as toxic or very toxic |
| | | | Classification of metabolite | not currently classified as toxic or very toxic |
| **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 | Required |
| | | | Predicted exposure (% of ADI) | 11.7% |
| | | | ADI based on | ADI for MT13 of 0.0034 mg/kg bw/d (3.4 µg/kg bw/d) |

* N/A: not applicable

### 10.3.1 STEP 1: Exclusion of degradation products of no concern

Could not be excluded

### 10.3.2 STEP 2: Quantification of potential groundwater contamination

| | | | |
|---|---|---|---|
| MT13<br>Hydroxy-terbuthylazine<br>Or 2-hydroxy terbuthylazine<br>GS 23158 |  | Max $PEC_{gw}$<br><br>Based on: | 14.95 µg/L<br><br>Focus PEARL 4.4.4 Hamburg |

Although the prediction of concentration in excess of 10µg/l may cause specific concerns in some MS, the RMS considers that these results represent conservative first tier exposure estimates only. The 2-hydroxy terbuthylazine metabolite was not detected above 0.1µg/l in the field leaching study performed in Northern Italy, even when other metabolites such as the desethyl-hydroxy terbuthylazine and the lysimeters leachate metabolites LM5 and LM6 were detected above 0.1µg/l as an annual average at some locations. In addition this metabolite was only detected in two wells (at < 0.05µg/l) in an extensive and targeted German groundwater monitoring program. In further recent groundwater monitoring studies in Italy in maize growing regions the 90th percentile concentration was only 0.03µg/l. On the basis of the additional information from field leaching and groundwater monitoring programs it is clear that the first tier FOCUS groundwater exposure assessments based on either the Notifier or co servative RMS approach represent a very conservative assessment and such high concentrations are unlikely to be encountered under realistic use conditions.

### 10.3.3 STEP 3: Hazard assessment – identification of relevant metabolites

#### 10.3.3.1 STEP 3, Stage 1: screening for biological activity

It was concluded that this metabolite is not herbicidally active.

#### 10.3.3.2 STEP 3, Stage 2: screening for genotoxicity

No evidence of genotoxicity was seen in a battery of studies in vitro**.**

#### 10.3.3.3 STEP 3, Stage 3: screening for toxicity

MT13 was found to be of low acute oral toxicity in the rat. A NOAEL of 3.4 mg/kg bw/d was determined for a 90-day toxicity study in the rat. An ADI for MT13 of 0.0034 mg/kg bw/d (3.4 µg/kg bw/d) can therefore be derived for MT13, based on the NOAEL from the 90-day study and applying a safety factor of 1000.

### 10.3.4 STEP 4: Exposure assessment – threshold of concern approach

MT13 is also predicted to exceed the 10 µg/L thresholds defined in the guidance document. However the additional monitoring data does indicate that the first tier FOCUS groundwater exposure assessment represents a conservative assessment for this metabolite and such high concentrations are unlikely to be encountered under realistic use conditions. However, as the first tier exposure assessment shows metabolites above the threshold of concern then refined risk assessments are provided for these metabolites in the following section, based on the conservative first tier FOCUSgw estimates.

### 10.3.5 STEP 5: Refined risk assessment

The maximum level of MT13 is predicted to be 14.95 µg/l on the basis of the conservative FOCUSgw modelling independently performed by the RMS. Where actual or predicted concentrations of a non-relevant metabolite in groundwater exceed 10 µg/L, no general guidance is provided in the Relevance of Metabolites in Groundwater document (SANCO/221/2000/rev:10-final 25 Feb 2003). Therefore, it is necessary to evaluate case by case, whether the requirements of Article 5 (1) of the Directive are still fulfilled and the active substance can be included in Annex I to the Directive. Such an assessment must consider the overall profile and use pattern of the substance and it must be based on strict precaution. Again on the basis of the additional information from field leaching and groundwater monitoring programs it is clear that the first tier FOCUS groundwater exposure assessments based on either the Notifiers or more conservative RMS approach represent a very conservative assessment and such high concentrations are unlikely to be encountered under realistic use conditions.
No new studies have been provided for MT13. Data on biological activity for MT13 have previously been provided and it was concluded that it was not herbicidally active. MT13 was found to be of low acute oral toxicity in the rat; no evidence of genotoxicity was seen in a battery of studies *in vitro*. A NOAEL of 3.4 mg/kg bw/d was determined for a 90-day toxicity study in the rat. An ADI for MT13 of 0.0034 mg/kg bw/d (3.4 µg/kg bw/d) can therefore be derived for MT13, based on the NOAEL from the 90-day study and applying a safety factor of 1000.

MT13 was identified as a minor rat metabolite (<1%) in the Oxon metabolism study (DAR 2010 Table B.6.19; M13), but was not identified as a metabolite in the Syngenta study. As this metabolite is potentially an intermediate in the formation of MT14 (desethylhydroxy-terbuthylazine, GS 28620), systemic exposure may be higher but is not possible to quantify. MT13 is not considered to be a relevant metabolite according to current EC guidance.

**Toxicological endpoints for MT13**

| metabolite | **Endpoint** | **Value (mg/kg bw/day)** | **Study** | **Safety factor** |
|---|---|---|---|---|
| MT13 Hydroxy-terbuthylazine Or 2-hydroxy terbuthy-lazine | Acceptable Daily Intake (ADI) | 0.0034 | 90-day toxicity study in the rat NOAEL | 1000 |

Intake (µg/kg bw/d) = 0.0267 L/kg bw/d x upper limit concentration of terbuthylazine metabolite [µg/L]

The following amounts for flufenacet metabolites by means of intake from drinking water and the corresponding ADI usages are calculated:

**Upper limit intake of MT13 through drinking water**

| **Metabolite** | Intake [µg/kg bw/d] expressed as parent equivalent | **Usage of ADI [%]** |
|---|---|---|
| MT13 Hydroxy-terbuthylazine Or 2-hydroxy terbuthy-lazine | 0.39916 | 11.7 |

From the long-term and short-term exposure calculations above it can be concluded that possible intakes of Hydroxy-terbuthylazine by means of drinking water do not present a consumer health concern. The calculations are based on several worst case assumptions.

Conclusion:

**In summary the metabolite MT 13 is considered to be biologically, toxicologically and ecotoxcologically non relevant.**

## 10.4 Relevance assessment of MT 14

**Summary:**

The relevance of the groundwater metabolite MT14 has already been assessed and the assessment agreed at EU level (DAR 2010 additional report) , 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 ). MT1 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 10.4-1: Summary of the relevance assessment for MT14 according to the Additional report 2010 DAR.**

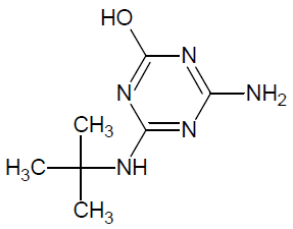
| | Assessment step | | Result of assessment | |
|---|---|---|---|---|
| | STEP 1 | | Metabolite of no concern? | Yes |
| Quantification of ground-water contamination | STEP 2 | | Max $PEC_{gw}$ | 3.45 µg/L |
| | | | Based on | FOCUS PEARL, Hamburg, 850 g a.s./ha, RMS simulation |
| Hazard assessment | 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; | Not toxic or very toxic ( T or T+) |
| | | | Classification of parent | not currently classified as toxic or very toxic |
| | | | Classification of metabolite | not currently classified as toxic or very toxic |
| 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 | Required |
| | | | Predicted exposure (% of ADI) | 0.9 % |
| | | | ADI based on | NOAEL from the 90-day study and applying a safety factor of 1000<br>ADI for MT14 of 0.0103 mg/kg bw/d (10.3 µg/kg bw/d) |

* N/A: not applicable

## 10.4.1 STEP 1: Exclusion of degradation products of no concern

Could not be excluded.

## 10.4.2 STEP 2: Quantification of potential groundwater contamination

| | | | |
|---|---|---|---|
| MT14<br>desethyl-hydroxyterbuthylazine<br>or<br>desethyl-2-hydroxy terbuthylazine<br>GS 28620 |  | Max $PEC_{gw}$<br><br>Based on: | 3.45 µg/L<br><br>Focus PEARL 4.4.4 Hamburg |

### 10.4.3 STEP 3: Hazard assessment – identification of relevant metabolites

#### 10.4.3.1 STEP 3, Stage 1: screening for biological activity

It was concluded that this metabolite is not herbicidally active.

#### 10.4.3.2 STEP 3, Stage 2: screening for genotoxicity

No evidence of genotoxicity was seen in a battery of studies in vitro.

#### 10.4.3.3 STEP 3, Stage 3: screening for toxicity

MT14 was found to be of low acute oral toxicity in the rat. A NOAEL of 10.3 mg/kg bw/d was determined for a 90-day toxicity study in the rat. An ADI for MT14 of 0.0103 mg/kg bw/d (10.3 µg/kg bw/d) can therefore be derived for MT14, based on the NOAEL from the 90-day study and applying a safety factor of 1000.

### 10.4.4 STEP 4: Exposure assessment – threshold of concern approach

**PECgw value is below TTC 0.75 µg/L, therefore STEP 5 of refined risk assessment is not necessary.**

### 10.4.5 STEP 5: Refined risk assessment

The maximum level of MT14 is predicted to be 3.97 µg/l. A refined risk assessment therefore needs to be performed, according to current EC guidance. Data on biological activity for MT14 have previously been provided and it was concluded that it was not herbicidally active. MT14 was found to be of low acute oral toxicity in the rat; no evidence of genotoxicity was seen in a battery of studies in vitro. A NOAEL of 10.3 mg/kg bw/d was determined for a 90-day toxicity study in the rat. An ADI for MT14 of 0.0103 mg/kg bw/d (10.3 µg/kg bw/d) can therefore be derived for MT14, based on the NOAEL from the 90-day study and applying a safety factor of 1000.
MT14 was identified as a rat metabolite in studies submitted by both Notifiers. It was identified as a metabolite in urine and faeces, although not at very high levels in the studies by Syngenta (≤7.8%; DAR Table B.6.18) and Oxon (4.41-11.6%, DAR Table B.6.19). MT14 is not considered to be a relevant metabolite according to current EC guidance.

**Toxicological endpoints for MT14**

| metabolite | **Endpoint** | **Value (mg/kg bw/day)** | **Study** | **Safety factor** |
|---|---|---|---|---|
| Metabolite MT14 (desethylhydroxy-terbuthylazine | Acceptable Daily Intake (ADI) | 0.0103 mg/kg bw/d | 90-day toxicity study in the rat NOAEL | 1000 |

Intake (µg/kg bw/d) = 0.0267 L/kg bw/d x upper limit concentration of terbuthylazine metabolite [µg/L]

The following amounts for terbuthylazine metabolites by means of intake from drinking water and the corresponding ADI usages are calculated:

**Upper limit intake of MT14 through drinking water**

| **Metabolite** | Intake [µg/kg bw/d] | **Usage of ADI [%]** |
|---|---|---|

| | | |
|---|---|---|
| | expressed as parent equivalent | |
| Metabolite MT14 (de-sethylhydroxy-terbuthylazine | 0.092 | 0.9% |

From the long-term and short-term exposure calculations above it can be concluded that possible intakes of desethylhydroxy-terbuthylazine by means of drinking water do not present a consumer health concern. The calculations are based on several worst case assumptions.

Conclusion:
**In summary the metabolite MT 14 is considered to be biologically, toxicologically and ecotoxcologically non relevant.**

## 10.5 Relevance assessment of LM1, LM2, LM3, LM4, LM5 and LM6

**Summary:**

In total seven novel leachate metabolites were characterised from the terbuthylazine lysimeters studies. Four of these metabolites (LM3, LM4, LM5 and LM6) represented the major identified fractions of the leachate and were subject to further analysis of their potential to contaminate groundwater using the FOCUSgw models. Two other metabolites were tentatively identified and considered to occur at or around the 0.1µg/l limit on an annual average basis. These two metabolites were coded LM1 and LM2 but have not been subject to further analysis via the FOCUSgw models. Since they represented more minor fractions of the lysimeters leachates compared with the other fractions (i.e. LM1 maximum annual average of 0.15µg/l and LM2 maximum annual average of 0.10µg/l) the RMS accepted the approach of the Notifiers to concentrate the additional quantification work using the FOCUS models on the major lysimeters fractions. In the opinion of the RMS the identity of the LM1 metabolite could only be tentatively assigned on the basis of the new mass spectral elucidation work evaluated in the fate section of the Additional Report (see Section B.8.2.3). In addition the LM2 metabolite occurred at a maximum annual average of 0.10µg/l. Nonetheless the non-relevance of LM1 and LM2 is discussed briefly below. The seventh novel leachate metabolite (LM7) was detected at a maximum annual average concentration of only 0.03µg/l has been excluded from further consid ration of its relevance.

For metabolite LM3 the Notifiers simulations gave PECgw values in the range from 0.448 to 2.21 µg/l. These values have been acceptably proposed to act as a surrogate for the likely worst case LM4 groundwater concentrations. For LM5 the Notifiers simulations gave PECgw values in the range from 0.157 to 2.08 µg/l. For LM6 the Notifiers simulations gave PECgw values in the range from 0.296 to 1.91 µg/l. In each case the highest PECgw values were found in the Hamburg scenario (simulating single applications of 844 g a.s./ha).

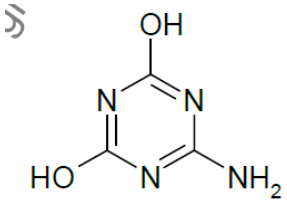
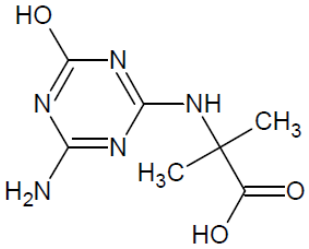
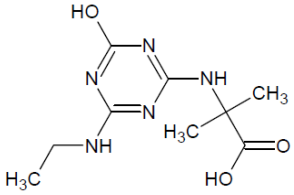
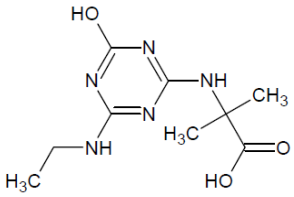
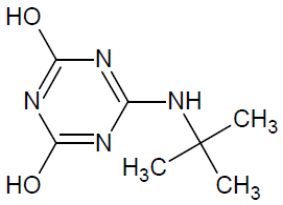
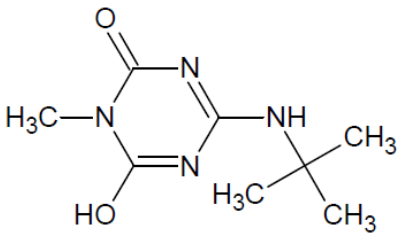
The predicted values for CSCD648241 (LM6) were noted to be in the same order of magnitude but higher than the residues measured in Germany in shallow groundwater wells (maximum 0.66 µg/l) and in Italy in piezometers under the treated field (annual average 1.3 µg/l). A similar result was found for GS16984 (LM5) where the maximum residue in German wells was 0.98 µg/l and the annual average in the Italian piezometers was 0.48 µg/l. CSCD692760 (LM3) residue data is only available for the German wells but again there was noted to be a close relationship between the predicted and measured (maximum 0.69 µg/L) values. Therefore, although some assumptions had to be made about the appropriate input values, the modelling does appear to be able to predict the fiel residues with reasonable accuracy.

Overall, despite the numerous uncertainties, the RMS chose to accept the groundwater simulations provided for the lysimeters metabolites in this case. The results of these simulations were broadly supported by results of the Italian field leaching study and the German groundwater monitoring program.

### 10.5.1 STEP 1: Exclusion of degradation products of no concern

**Could not be excluded**

### 10.5.2 STEP 2: Quantification of potential groundwater contamination

| | | | |
|---|---|---|---|
| LM1<br>MT24 |  | Based on, lysimeter studies: | 0.15µg/l |
| LM 2<br>MT28 |  | Based on lysimeter: | 0.10 µg/l |
| LM 3<br>SM9<br>CSCD692760<br>SYN546009 |  | Based on lysimeter: | Notifiers simulations gave PECgw values in the range from 0.448 to 2.21 µg/l. |
| LM4<br>SM4<br>CSAA404949<br>GS40436 |  | Max $PEC_{gw}$<br><br>Based on LM3: | Notifiers simulations gave PECgw values in the range from 0.448 to 2.21 µg/l. |
| LM5<br>MT23<br>SM12<br>GS 16984 |  | Max $PEC_{gw}$<br><br>Based on: | Notifiers simulations gave PECgw values in the range from 0.157 to 2.08 µg/l |
| LM6<br>SM6<br>CSCD648241<br>SYN545666 |  | Max $PEC_{gw}$<br><br>Based on: | Notifiers simulations gave PECgw values in the range from 0.296 to 1.91 µg/l |

## 10.5.3 STEP 3: Hazard assessment – identification of relevant metabolites

### 10.5.3.1 STEP 3, Stage 1: screening for biological activity

Biological activity testing studies for five of the six lysimeter metabolites found at > 0.1 µg/L have also been submitted as part of this resubmission (i.e. LM2 to LM6). The only exception was G035713 (LM1) but the Notifiers stated that this is a degradate of GS16984 (LM5, MT23) which has been shown to have no biological activity.

The biological activi y of the metabolites CSCD648241 (LM6), CSCD692760 (LM3), GS16984 (LM5, MT23), CSAA036479 (LM2) and CSAA404949 (LM4) is less than 50% of the parent molecule when applied at a dose at which the parent demonstrates good herbicidal activity on key species. On this basis these metabolites should not be considered as being 'relevant' in terms of biological activity.

### 10.5.3.2 STEP 3, Stage 2: screening for genotoxicity

**Metabolite LM1**

The Notifiers have studies ongoing with this metabolite. The metabolite does not possess any structural alerts for genotoxicity according to DEREK and does not contain any additional functional groups that are not present in terbuthylazine or its metabolites (including metabolites MT1, MT13, MT14 and M20 which have been tested for genotoxicity). A mammalian gene mutation test is also available but was concluded too late to be included in the resubmiss on so has not been evaluated.

**Metabolite LM2**

The Notifiers have studies ongoing with this metabolite. The metabolite does not possess any structural alerts for genotoxicity according to DEREK and does not contain any additional functional groups that are not present in terbuthylazine or its metabolites (including metabolites MT1, MT13, MT14 and M20 which have been tested for genotoxicity). An Ames test is also available but was concluded too late to be included in the resubmission so has not been considered.

**Metabolite LM3**

The Notifiers have provided an Ames assay with this metabolite and it is negative. The metabolite does not possess any structural alerts for genotoxicity according to DEREK and does not contain any additional functional groups that are not present in terbuthylazine or its metabolites (including metabolites MT1, MT13, MT14 and M20 which have been tested for genotoxicity). A mammalian gene mutation test is also available but was concluded too late to be included in the resubmission so has not been considered.

**Metabolite LM4**

The metabolite does not possess any structural alerts for genotoxicity according to DEREK and does not contain any additional functional groups that are not present in terbuthylazine or its metabolites (including metabolites MT1, MT13, MT14 and M20 which have been tested for genotoxicity) and is structurally very similar to MT13 and MT14. An Ames assay is also available but was concluded too late to be included in the resubmission so have not been considered.

**Metabolite LM5 (GS 16984)**

The Notifiers have provided an Ames assay with this compound and it is negative. The metabolite does not possess any structural alerts for genotoxicity according to DEREK and does not contain any additional functional groups that are not present in terbuthylazine or its metabolites (including metabolites MT1,

MT13, MT14 and M20 which have been tested for genotoxicity). An in-vitro chromosome aberration test and a mammalian gene mutation test are also available but were concluded too late to be included in the resubmission so have not been considered.

**Metabolite LM6**

In the resubmission package the Notifiers have provided a reverse mutation assay, a mouse lymphoma assay, in vitro chromosome aberration study in Human Lymphocytes, and an in vivo rat bone marrow micronucleus test. Although positive at cytotoxic levels in the gene mutation assay overall it is conside ed non-genotoxic. The metabolite does not possess any structural alerts for genotoxicity according to DEREK and is structurally similar to MT13 and MT14.

### 10.5.3.3 STEP 3, Stage 3: screening for toxicity

**Metabolite LM1**

LM1 also known as ammelide is a mammalian metabolite of melamine. Melamine has a long history of use in a range of products i.e. in combination with formaldehyde to produce melamine resin as durable thermosetting plastics, and melamine foam, a polymeric cleaning product. Other end products include countertops, fabrics, glues and flame retardants. It is also a major component of pigment yellow 150 (colorant for inks and plastics), fertilizers, and derivatives of arsenical drugs for the treatment of African sleeping sickness (trypanosomiasis).

Melamine is a metabolite of cyromazine (an Annex I listed active substance see EFSA Scientific Report (2008) 168, 1-94 Conclusion on the peer review of cyromazine). The RMS produced an extensive review of the published literature on melamine and concluded melamine was found to have no toxicological relevance for groundwater according to the guidance document on groundwater metabolites. The RMS proposed to set an ADI of 0.063 mg/kg bw/day for melamine based on the review, however the meeting considered that the ADI of the parent (cyrom zine) should be considered relevant for melamine risk assessment. The ADI for cyromazine was set at 0.06 mg/kg bw/day. Based on this it is likely toxicity of metabolite LM1 is less than that of terbuthylazine and the tested metabolites.

**Metabolite LM2**

LM2 contains an additional carboxylic acid functional group when compared to terbuthylazine and is a hydroxyl metabolite. Also it does not contain any additional functional groups that are not present in terbuthylazine or its metabolites (including metabolites MT1, MT13, MT14 and M20 which have been tested for genotoxicity). It can be reasonably predicted that the toxicity of metabolite LM2 is less than that of terbuthylazine and the tested metabolites.

**Metabolite LM3**

Metabolite LM3 contains an additional carboxylic acid functional group (when compared to terbuthylazine and the tested metabolites), but in this respect is structurally similar to the carboxylic acid metabolites MT5, MT8 (GS 33022) and MT10 (GS 31398). It can be reasonably predicted that the toxicity of metabolite LM3 is less than that of terbuthylazine and the tested metabolites**.**

**Metabolite LM4**

The metabolite does not contain any additional functional groups that are not present in terbuthylazine or its metabolites (including metabolites MT1, MT13, MT14 and M20 which have been tested for genotoxicity) and is structurally very similar to MT13 and MT14 which have been tested for toxicity. Deleted comment assessment relies on consumer assessment below.

**Metabolite LM5 (GS 16984)**

The metabolite does not contain any additional functional groups that are not present in terbuthylazine or its metabolites (including metabolites MT1, MT13, MT14 and M20 which have been tested for genotoxicity). It can be reasonably predicted that the toxicity of metabolite LM5 is less than that of terbuthylazine.

**Metabolite LM6**

The metabolite is structurally similar to MT13 and MT14. It can be reasonably predicted that the toxicity of metabolite LM6 is less than that of terbuthylazine.

### 10.5.4 STEP 4: Exposure assessment – threshold of concern approach

For those metabolites for which the exposure assessment shows they are below the threshold of concern which is given in the Guidance Document as 0.75 µg/L they can be determined to be non relevant at Step 4.

The concentration of the metabolites LM3, LM4, LM5 and LM6 are predicted to exceed the 0.75 µg/L. LM1 and LM2 are not predicted to exceed 0.75µg/L and therefore are excluded from further consideration due to their non-relevance as demonstrated above. However, as the first tier exposure assessment shows metabolites above the threshold of concern then refined risk assessments are provided for these metabolites in the following section, based on the conservative first tier FOCUSgw estimates.

### 10.5.5 STEP 5: Refined risk assessment

**Metabolite LM3**

The maximum level of LM3 is predicted to be 2.21µg/l. A refined risk assessment therefore needs to be performed, according to current EC guidance. Data on biol gical activity for this metabolite have been provided and demonstrated it is less than 50% of the parent molecule when applied at a dose at which the parent demonstrates good herbicidal activity on key species. The Notifiers have provided an Ames assay with this metabolite and it is negative. The metabolite does not possess any structural alerts for genotoxicity according to DEREK and does not contain any additional functional groups that are not present in terbuthylazine or its metabolites (including metabolites

MT1, MT13, MT14 and M20 which have been tested for genotoxicity). Metabolite LM3 contains an additional carboxylic acid functional groupRegistration(when compared to terbuthylazine and the tested metabolites), but in this respect structurally similar to

the carboxylic acid metabolites MT5, MT8 (GS 33022) and MT10 (GS 31398). It can be reasonably predicted that the toxicity of metabolite LM3 is less than that of terbuthylazine and the tested metabolites.

Using the highest predicted groundwater concentration of 2.21 µg/l. The maximum predicted consumer intake is equivalent to 1.8% of the proposed ADI for terbuthylazine of 0.004 mg/kg bw/d (4 µg/kg bw/d) and is therefore considered to be acceptable.

**Metabolite LM4**

Results for LM3 have been proposed to act as a surrogate for LM 4 (CSAA404949) therefore maximum level of MT4 is predicted by modelling to be 2.21 µg/l. A refined risk assessment therefore

needs to be performed, according to current EC guidance. Data on biological activity for this metabolite have been provided and demonstrated it is less than 50% of the parent molecule when applied at a dose at which the parent demonstrates good herbicidal activity on key species. The metabolite does not possess any structural alerts for genotoxicity according to DEREK and does not contain any additional functional groups that are not present in terbuthylazine or its metabolites (including metabolites MT1, MT13, MT14 and M20 which have been tested for genotoxicity) and is structurally very similar to MT13 and MT14.

Using the highest predicted groundwater concentration of 2.21 µg/l. The maximum predicted consumer intake is equivalent to 1.8% of the proposed ADI for terbuthylazine of 0.004 mg/kg bw/d (4 µg/kg bw/d) and is therefore considered to be acceptable.

**Metabolite LM5 (GS 16984)**

The maximum level of LM5 is predicted to be 2.08 µg/l. A refined risk assessment therefore needs to be performed, according to current EC guidance. Data on biological activity for this metabolite have been provided and demonstrated it is less than 50% of the parent molecule when applied at a dose at which the parent demonstrates good herbicidal activity on key species. The Notifiers have provided an Ames assay with this compound and it is negative. The metabolite does not possess any structural alerts for genotoxicity according to DEREK and does not contain any additional functional groups that are not present in terbuthylazine or its metabolites (including metabolites MT1, MT13, MT14 and M20 which have been tested for genotoxicity). It can be reasonably predicted that the toxicity of metabolite LM5 is less than that of terbuthylazine.

Using the highest predicted groundwater concentration of 2.08 µg/l. The maximum predicted consumer intake is equivalent to 1.7% of the proposed ADI for terbuthylazine of 0.004 mg/kg bw/d (4 µg/kg bw/d) and is therefore considered to be acceptable.

**Metabolite LM6**

The maximum level of LM6 is predicted by modelling to be Registration 1.91 µg/l. A refined risk assessment therefore needs to be performed, according to current EC guidance. Data

on biological activity for this metabolite have been provided and demonstrated it is less than 50% of the parent molecule when applied at a dose which the parent demonstrates good herbicidal activity on key species. On this basis these metabolites should not be considered as being ‘relevant’ in terms of biological activity. In the resubmission package the Notifiers have provided a reverse mutation assay, a mouse lymphoma assay, in vitro chromosome aberration study in Human Lymphocytes, and an in vivo rat bone marrow micronucleus test. Although positive at cytotoxic levels in the gene mutation assay overall it is considered non-genotoxic. The metabolite does not possess any structural alerts for genotoxicity according to DEREK and is structurally similar to MT13 and MT14. It can be reasonably predicted that the toxicity of metabolite LM6 is less than that of terbuthylazine.

Using the highest predicted groundwater concentration of 1.91 µg/l. The maximum predicted consumer intake is equivalent to 1.6% of the proposed ADI for terbuthylazine of 0.004 mg/kg bw/d (4 µg/kg bw/d) and is therefore considered to be acceptable.

## 10.6 Relevance assessment of RPA 202248– Isoxaflutole metabolite

| **Evaluator’s comments:** |
|---|
| 1. The metabolite RPA 202248 **has pesticidal activity.** It is the major rat metabolite of low acute oral toxicity and negative results of Ames test. |

2. Since isoxaflutole is classified as Repr. 2, H361d, convincing evidence must be provided to demonstrate that the metabolite RPA 203328 does not qualify for the same classification. **The available toxicological data were not sufficient to exclude reproduction toxicity of metabolite RPA 202248**.
3. As the pesticidal active and mammalian toxicologically relevant, the metabolite RPA 202248 should not exceed drinking water limit of 0.1 µg/L.
4. Taking into account that the appl. rate amounts to 100 g of the active substance used once each year and the max. $PEC_{gw}$ amounts to 1.154 µg/L, the metabolite RPA 202248 causes significant consumer risk for human health and does not meet the conditions of product approval.
5. Taking into account that the max. appl. rate amounts to **80 g/ha** of the active substance used **once every three years, the max. $PEC_{gw}$ of RPA 202248 is below 0.1 µg/L and does not cause consumer risk for human health.**

Conclusions: **Only the application rate of 80 g of the product /ha can be accepted. The product can be used once every three years.**

**Summary:**

The relevance of the groundwater metabolite RPA202248 has already been assessed and the assessment agreed at EU level (see RAR isoxaflutole Volume 1 – Level 2 2016) , 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 ). RPA202248 is not considered relevant according to the criteria laid down in the EC guidance document SANCO/221/2000 – rev.10.

**According to the RAR isoxaflutole Volume 1 – Level 2 2016:**
The maximum $PEC_{gw}$ for RPA 202248 is 0.272 µg/L for annual application, 0.128 µg/L for 1:2 years rotation and 0.099 µg/L for a 1:3 year rotation. No information on the toxicological relevance of this metabolite is available, therefore just a 1:3 year rotation provide a safe use in all scenarios while for annual application just in 5 of the 9 scenarios modelled, the metabolite does not leach.

**Summary of the results of toxicity studies for RPA 202248**

| Type of test, species | Result | Acceptability | Reference* |
|---|---|---|---|
| Acute oral toxicity (male & female Sprague-Dawley rats) | LD50 > 5000 mg/kg bw | | -, (1995a) |
| Bacterial mutagenicity ( *Salmonella typhimurium* ) | Negative | | Percy, 1995 |

The metabolite is not toxicological relevant relevant.

## 10.7 Relevance assessment of– Isoxaflutole metabolite

**Evaluator's comments:**

1. According to EFSA Journal 2016;14(2):4416, the consumer risk assessment resulting from consumption of drinking water could not be finalized although the nature of the residues in drinking water following water treatment had not been addressed. Taking into account toxicological data, the metabolite RPA 203328 possess low oral acute toxicity and has no genotoxic potential.
2. Since isoxaflutole is classified as Repr. 2, H361d, convincing evidence must be provided to demonstrate that the metabolite RPA 203328 does not qualify for the same classification. Taking into account the mechanism of reproduction toxicity of the parent substance, the tox-

icological data obtained for RPA 203328 indicate lack of such effect.

3. The $PEC_{gw}$ for this metabolite exceeds 0.75 µg/L. Thus, the consumer risk is required.
4. Taking into account the ADI value for RPA 203328 (0.8 mg/kg bw) and max. $PEC_{gw}$ which amounts to 3.867 µg/L, the results of risk calculations for RPA 203328 are as follows:

| | Exposure (µg/kg b.w./d) | % ADI (parent substance) |
|---|---|---|
| Adults ($70^1/60^2$ kg b.w.) | 0.129/0.11 | **0.016/0.014** |
| Toddlers ($12^1/10^2$ kg b.w.) | 0.32/0.39 | **0.04/0.048** |
| Infants ($5^{1,2}$ kg b.w.) | 0.58 | **0.073** |

The results of consumer risk calculations in regards to the metabolite RPA 203328 indicate that the use of CHR/H/TERIZ 650 WG according to the list of intended uses presented in GAP Table, **causes no significant risk for health of the adults, toddlers and infants.**

[1]*According to EFSA Journal 2012;10(3):2579, Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data.*

[2]*WHO Guidelines for drinking-water quality: fourth edition incorporating the first addendum, 2017*

**Summary:**

The relevance of the groundwater metabolite RPA 203328 has already been assessed and the assessment agreed at EU level (see RAR isoxaflutole Volume 1 – Level 2 2016) , 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 ). RPA203328 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 10.7-1: Summary of the relevance assessment for 203328 according to the RAR isoxaflutole Volume 1 – Level 2 2016**

| | **Assessment step** | | **Result of assessment** | |
|---|---|---|---|---|
| | STEP 1 | | Metabolite of no concern? | Yes |
| **tion of groun dwa- ter con** | STEP 2 | | Max $PEC_{gw}$ | ~~2.824~~ 3.867 µg/L |
| | | | Based on | FOCUS PEARL, Hamburg |
| **Haz- ard as- sess ment** | 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; | Not toxic or very toxic ( T or T+) |
| | | | Classification of parent | not currently classified as toxic or very toxic |
| | | | Classification of metabolite | not currently classified as toxic or very toxic |
| **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 | Required |
| | | | Predicted exposure (% of ADI) | 18.3 % |
| | | | ADI based on | ADI 0.02 mg/kg bw/day |

**According to the RAR isoxaflutole Volume 1 – Level 2 2016:**
The benzoic acid metabolite (RPA 203328) shows PECgw values above 0.1 µg/L in tall types of application, consequently, its relevance has to be considered in terms of biological, genotoxicological and toxicological activity according to EU Guidance Document SANCO/221/2000, rev. 10 (2003). The maximum PECgw for RPA 203328 was 2.824 µg/L when isoxaflutole is applied every year

The herbicidal activity of RPA 203328 was tested in greenhouse tests against a representative range of monocotyledonous and dicotyledonous crop and weed species RPA 203328 was tested at rates ranging from 62 g/ha to 4000 g/ha. It was applied pre-emergence for identification of effects on germination, emergence and early development on test plants after possible root or hypocotyl uptake. It also was applied post-emergence for identification of possible effects on more advanced stages of development of test plants after foliar or root uptake. In neither of the two tests of RPA 203328 were any symptoms of herbicidal activity on any of the species tested observed. It is concluded that RPA 203328 does not have any herbicidal activity at rates close to the use rate of the parent compound (62 g/ha RPA 203328 compares to 83 g/ha isoxaflutole calculated as molar equivalents) and also not at higher use rates (250 – 4000 g/ha), which will not be reached under practical conditions in registered agricultural use patterns. It is also important to mention that the herbicidal activitywas determined in greenhouse tests where weeds and crops are more susceptible to damage than under natural conditions in the field.
In the same tests, isoxaflutole exhibited strong herbicidal activity even at the lowest rate tested (16 g/ha) against a wide variety of grasses and broadleaf plants when applied pre- or post-emergence .
These results confirm that RPA 203328 has no herbicidal properties at dose rates much greater than the isoxaflutole labelled rate. The proposed target of less than 50 % activity compared to the parent compound and therefore the trigger value given in revision 10 is clearly met.

The mutagenic potential of RPA 203328 was assessed in an Ames assay using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA 1537 (Percy, 1995 M-170821-01-1). The material was tested in triplicate at concentrations of 100, 250, 500, 1000, 2500 and 5000 µg/plate in the presence and absence of a metabolic activation system (S9 mix) obtained from livers of rats pre-treated with Aroclor 1254. A cytotoxic effect, as indicated by a thinning of the bacterial background lawn, was noted on a majority of plates containing 2500 and 5000 µg/plate. RPA 203328 did not induce any concentration-dependent, significant increases in the numbers of revertants of any strain at any of the concentrations studied. RPA 203328 was considered non-mutagenic under the conditions of this test.
The mutagenic potential of RPA 203328 was also assessed in two mutagenicity studies using Chinese hamster ovary cells (Murli, 1998 M-157884-01-1, and Cifone, 1998 M-189726-01-2), in which the material was tested at concentrations of up to 2700 µg/ml in both the presence and the absence of metabolic activation. There was some cytotoxicity observed in some cultures, but there was no indication of muta-

genic activity of RPA 203328 at any concentration. RPA 203328 was therefore considered non-mutagenic under the conditions of this test.

The clastogenicity of RPA 203328 was tested in vivo in a mouse micronucleus assay (Curry, 1998 M-211247-01-1), in which the test substance was administered by oral gavage to male mice at doses of up to 2000 mg/kg bw/. There was no alteration in the incidence of micronucleated polychromatic erythrocytes after administration of RPA 203328, indicating that the test substance is not clastogenic in the test system.
In a battery of genotoxicity tests conducted using isoxaflutole as the test substance, no evidence of mutagenicity was noted either with or without metabolic activation. The presence of the metabolic activation system would be expected to convert isoxaflutole to its two primary metabolites, RPA 202248 and RPA 203328. The formation of RPA 203328 is an oxidation reaction typically carried out by hepatic enzymes such as the cytochrome P-450 family.
These enzymes and others (for example, hydrolytic enzymes) would be present in the standard rat liver S-9 mix used as the metabolic activation system in *in vitro* mutagenicity assays. As the mutagenicity studies on isoxaflutole using a metabolic activation system were negative, one can infer that the metabolites of isoxaflutole are not mutagenic. The findings of the Ames assay conducted with RPA 203328 itself support this assumption.
Based on the battery of genotoxicity studies conducted it can be shown that there is no genotoxic potential of the metabolite RPA 203328.

RPA 203328 has very low acute toxicity. The LD50 value for this compound was determined to be greater than the limit value of 5000 mg/kg (Bigot, 1995 M-170815-01-1). Additionally, the signs observed during this study are not different from those observed in acute oral toxicity studies performed with other substances, in which the LD50 is greater than the limit dose of 5000 mg/kg.
In the 28-Day Repeated-Dose study with RPA 203328, there were no mortalities, and no treatment-related clinical signs were observed during the study. No effects were seen in body weight, food consumption, or ophthalmological parameters (Dange, 1995 M-170705-01-1). No changes were noted in hematology, clinical chemistry, or urinalysis. At necropsy, no changes were observed macroscopically or in organ weights. Upon histological examination, no changes attributable to RPA 203328 administration were observed in any of the 46 tissues examined. Specifically, there were no signs of hepatic necrosis, hypertrophy, changes in liver enzymes such as AST and ALT, or other adverse findings. The No Observed Effect Level was found to be 15,000 ppm, indicating that the toxicity of RPA 203328 is much lower than the parent and is unlikely to be associated with long-term liver effects. In addition, the lack of liver effects in this study (coupled with the high water solubility of RPA 203328) indicates that RPA 203328 is unlikely to induce hepatic cytochrome P-450 enzymes. This is in contrast to the parent isoxaflutole, which caused hepatocellular hypertrophy, increased liver weight, and induction of both cytochrome P450 and Phase II enzymes.
Furthermore, in the 90-day study conducted with RPA 203328 (Bigot, 1998 M-240662-01-1), there were no mortalities, clinical signs, or changes in body weight or body weight gain in either males or females, and no effects on either hematological or clinicochemical parameters. There were also no effects of administration of RPA 203328 at doses of up to 12000 ppm on organ weights or histopathology in either sex. At necropsy there were some findings (dark or yellowish liver, marked lobular liver, and / or dark kidneys) noted in some animals, however there was no consistency of findings between sexes and there were no histopathological correlates to these gross findings. The No Observed Adverse Effect level was found to be 12000 ppm (768.9 mg/kg bw/day in males, 952.4 mg/kg bw/day in females), in contrast to the 90-day study conducted with the parent isoxaflutole, in which the NOEL was established at 3 mg/kg bw/day. Isoxaflutole itself has been labelled with the Cat. 3, R63 based on results of the rat developmental toxicity study, which included decreased fetal body weight and ossification and increased incidence of subcutaneous edema and hemorrhage. The proposed mechanism by which isoxaflutole causes these effects in the developing fetus is the induction of Phase II enzymes including UDPGT, resulting in a decrease in circulating thyroid hormone levels in the dam, leading to delayed but not decreased fetal development. The lack of any effect on the liver with 28-day administration of RPA 203328, including liver weight or hypertrophy, indicates that RPA 203328 is highly unlikely to induce Phase II enzymes, will not decrease circulating thyroid hormone levels, and therefore will not delay fetal development.

A developmental toxicity study was conducted in the rat with RPA 203328 (Repetto-Larsay, 1999 M-189848-01-1), using doses of 0, 75, 250, and 750 mg/kg bw/day administered by oral gavage on gestation days 6 through 20. Maternal food consumption and body weight gain was decreased at 250 and 750 mg/kg bw/day. Gestation rate, implantation rate, pre- and post-implantation mortality, the number of viable young, and sex ratio were unaffected by administration of RPA 203328. At examination of the fetuses, there was no effect of treatment on fetal body weight, or on external, visceral, or skeletal observations. The maternal NOAEL was 75 mg/kg bw/day, while the fetal NOAEL was 750 mg/kg bw/day, the highest dose tested.
Therefore, RPA 203328 is not capable of causing the fetal effects observed with isoxaflutole, and does not warrant any reproductive classification.
Following the provisions given under Step 3 RPA 203328 cannot be considered as relevant. An exposure and/or risk assessment has to be conducted. It has been shown in a risk-based approach, combining several worst case assumptions, that long-term and short-term intakes of residues of RPA 203328 via food and drinking water is unlikely to present a public health concern.

### 10.7.1 STEP 1: Exclusion of degradation products of no concern

Could not be excluded

### 10.7.2 STEP 2: Quantification of potential groundwater contamination

Based on FOCUS PEARL, Hamburg PECgw=12.43µg/L

### 10.7.3 STEP 3: Hazard assessment – identification of relevant metabolites

#### 10.7.3.1 STEP 3, Stage 1: screening for biological activity

The herbicidal activity of RPA 203328 was tested in greenhouse tests against a representative range of monocotyledonous and dicotyledonous crop and weed species. RPA 203328 was tested at rates ranging from 62 g/ha to 4000 g/ha. It was applied pre-emergence for identification of effects on germination, emergence and early development on test plants after possible root or hypocotyl uptake. It also was applied post-emergence for identification of possible effects on more advanced stages of development of test plants after foliar or root uptake. In neither of the two tests of RPA 203328 were any symptoms of herbicidal activity on any of the species tested observed. It is concluded that RPA 203328 does not have any herbicidal activity at rates close to the use rate of the parent compound (62 g/ha RPA 203328 compares to 83 g/ha isoxaflutole calculated as molar equivalents) and also not at higher use rates (250 – 4000 g/ha), which will not be reached under practical conditions in registered agricultural use patterns. It is also important to mention that the herbicidal activity was determined in greenhouse tests where weeds and crops are more susceptible to damage than under natural conditions in the field.
**These results confirm that RPA 203328 has no herbicidal properties at dose rates much greater than the isoxaflutole labelled rate. The proposed target of less than 50 % activity compared to the parent compound and therefore the trigger value given in revision 10 is clearly met.**

### 10.7.3.2 STEP 3, Stage 2: screening for genotoxicity

The mutagenic potential of RPA 203328 was assessed in an Ames assay using Salmonella typhimurium strains TA98, TA100, TA1535 and TA 1537 [2]. The material was tested in triplicate at concentrations of 100, 250, 500, 1000, 2500 and 5000 µg/plate in the presence and absence of a metabolic activation system (S9 mix) obtained from livers of rats pre-treated with Aroclor 1254. A cytotoxic effect, as indicated by a thinning of the bacterial background lawn, was noted on a majority of plates containing 2500 and 5000 µg/plate. RPA 203328 did not induce any concentration-dependent, significant increases in the numbers of revertants of any strain at any of the concentrations studied. RPA 203328 was considered non-mutagenic under the conditions of this test.

In a battery of mutagenicity tests conducted using isoxaflutole as the test substance, no evidence of mutagenicity was noted either with or without metabolic activation. The presence of the metabolic activation system would be expected to convert isoxaflutole to its two primary metabolites, RPA 202248 and RPA 203328. The formation of RPA 203328 is an oxidation reaction typically carried out by hepatic enzymes such as the cytochrome P-450 family.

These enzymes and others (for example, hydrolytic enzymes) would be present in the standard rat liver S-9 mix used as the metabolic activation system in in vitro mutagenicity assays. As the mutagenicity studies on isoxaflutole using a metabolic activation system were negative, one can infer that the metabolites of isoxaflutole are not mutagenic. The findings of the Ames assay conducted with RPA 203328 itself support this assumption.

**Based on the battery of mutagenic studies conducted it can be shown that there is no genotoxic potential of the metabolite RPA 203328.**

### 10.7.3.3 STEP 3, Stage 3: screening for toxicity

RPA 203328 has very low acute toxicity. The $LD_{50}$ value for this compound was determined to be greater than the limit value of 5000 mg/kg [3]. Additionally, the signs observed during this study are no different from those observed in acute oral toxicity studies performed with other substances, in which the $LD_{50}$ is greater than the limit dose of 5000 mg/kg.

In the 28-Day Repeated-Dose study with RPA 203328, there were no mortalities, and no treatment-related clinical signs were observed during the study. No effects were seen in body weight, food consumption, or ophthalmological parameters [4]. No changes were noted in hematology, clinical chemistry, or urinalysis. At necropsy, no changes were observed macroscopically or in organ weights. Upon histological examination, no changes attributable to RPA 203328 administration were observed in any of the 46 tissues examined. Specifically, there were no signs of hepatic necrosis, hypertrophy, changes in liver enzymes such as AST and ALT, or other adverse findings. The No Observed Effect Level was found to be 15,000 ppm, indicating that the toxicity of RPA 203328 is much lower than the parent and is unlikely to be associated with long-term liver effects. In addition, the lack of liver effects in this study (coupled with the high water solubility of RPA 203328) indicates that RPA 203328 is unlikely to induce hepatic cytochrome P-450 enzymes. This is in contrast to the parent isoxaflutole, which caused hepatocellular hypertrophy, increased liver weight, and induction of both cytochrome P450 and Phase II enzymes.

Isoxaflutole itself has been labelled with the Cat. 3, R63 based on results of the rat developmental toxicity study, which included decreased fetal body weight and ossification and increased incidence of subcutaneous edema and hemorrhage. The proposed mechanism by which isoxaflutole causes these effects in the developing fetus is the induction of Phase II enzymes including UDPGT, resulting in a decrease in circulating thyroid hormone levels in the dam, leading to delayed but not decreased fetal development. The lack of any effect on the liver with 28-day administration of RPA 203328, including liver weight or hy-

pertrophy, indicates that RPA 203328 is highly unlikely to induce Phase II enzymes, will not decrease circulating thyroid hormone levels, and therefore will not delay fetal development.
**Therefore, RPA 203328 is not capable of causing the fetal effects observed with isoxaflutole, and does not warrant any reproductive classification.**

**Following the provisions given under Step 3 RPA 203328 can not be considered as relevant. An exposure and/or risk assessment has to be conducted**.

### 10.7.4 STEP 4: Exposure assessment – threshold of concern approach

According to provisions made under Step 4, the metabolite RPA 203328 although not considered as relevant may exceed the Threshold of Concern of 0.75 µg/l in the different FOCUS maize scenarios as estimated by the groundwater simulations using FOCUS PEARL 4.4.4 and therefore is subject to a refined assessment in Step 5.

### 10.7.5 STEP 5: Refined risk assessment

**Toxicological endpoints for Isoxaflutole**

| Active substance | **Endpoint** | **Value (mg/kg bw/day)** | **Study** | **Safety factor** | **Reference** |
|---|---|---|---|---|---|
| ~~Mesotrione~~ Isoxaflutole | Acceptable Daily Intake (ADI) | 0.02 | 2 y rat study | 100 | Sanco/3136/99-Final 7 April 2003 |
| | Acute Reference Dose (ARfD) | Not allocated (not necessary) | | | |

**Table 10.11-2 Conversion of gw concentration expressed as metabolite to parent equivalents**

| **Metabolite** | Estimated upper limit gw concentration expressed as metabolite [µg/L] | Estimated upper limit gw concentration expressed as parent equivalent [µg/L] |
|---|---|---|
| RPA 203328 | 12.43 | 9.28 |

Molar mass isoxaflutole 359.32 g/mol
Molar mass RPA 203328 268.22 g/mol

When outlining the assessment of exposure the SANCO document 221/2000 rev 10 considers a toxicological threshold of 0.75 µg/kg bw/d or 1.5 µg/person/day assuming a consumption of 2 liters of water per day which is a conservative value also recommended by WHO (1994) (cf. to step 4 of SANCO 221/2000). Based on these considerations an intake of 2 liters of water/day corresponds to 26.7 mL/kg bw/d or 0.0267 L/kg bw/d. Hence, the intake of residues through drinking water is calculated using the following equation:

Intake (µg/kg bw/d) = 0.0267 L/kg bw/d x upper limit concentration of isoxaflutole metabolite [µg/L]

The following amounts for flufenacet metabolites by means of intake from drinking water and the corresponding ADI / ARfD usages are calculated:

**Table 10.2.5-3 Upper limit intake of RPA 203328 through drinking water**

| **Metabolite** | Intake [µg/kg bw/d] expressed as parent equivalent | **Usage of ADI [%]** |
|---|---|---|
| isoxaflutle | 0.2479 | 1.3 |

Following the criteria outlined in revision 10 "Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive 91/414/EEC", RPA 203328 fully qualifies as a non-relevant metabolite of isoxaflutole.

It has been demonstrated that the Isoxaflutole metabolite RPA 203328

- Has very low herbicidal activity (<< 50 % compared to the parent compound);
- Is not genotoxic;
- Shows very low acute and subchronic toxicity with no mechanistic link to the effects seen in the parent studies.

Besides,

- has no relevant toxicity for fish, daphnid and algae;
- is not acutely toxic to earthworms and have no significant effect on carbon and nitrogen mineralisation up to 0.9 mg/kg soil;

Thus RPA 203328 passes all three hazard assessment criteria.

It has been shown in a risk-based approach that RPA 203328 as a metabolite formed from isoxaflutole provides high Margins of Safety for different consumer groups (greater than $3.1 \times 10^6$) and poses no toxicological risk via dietary exposure. Besides RPA 203328 has no relevant toxicity for fish, daphnid and algae and no risk is expected for soil organisms and functions.

Following this rationale, the parent compound can be included in Annex 1 of the Directive 91/414/EEC.

The conclusion that RPA203328 is not relevant is confirmed by the SCP in the SCP opinion on isoxaflutole of 30/01/200

## 10.8 Relevance assessment of MNBA

**Summary:**

The relevance of the groundwater metabolite MNBA has already been assessed and the assessment agreed at EU level (see RARmesotrione Volume 1 – Level 2 2015) , 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 ). MNBA 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 10.12-1: Summary of the relevance assessment for MNBA according to the RARmesotrione Volume 1 – Level 2 2015**

| | Assessment step | | Result of assessment | |
|---|---|---|---|---|
| | STEP 1 | | Metabolite of no concern? | Yes |
| tion of groun dwa- ter con | STEP 2 | | Max $PEC_{gw}$ | 0.121 µg/L |
| | | | Based on | FOCUS PELMO, Hamburg |
| 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; | Not toxic or very toxic ( T or T+) |
| | | | Classification of parent | not currently classified as toxic or very toxic |
| | | | Classification of metabolite | not currently classified as toxic or very toxic |
| 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 | N/A |
| | | | Predicted exposure (% of ADI) | N/A |
| | | | ADI based on | N/A |

Two models PEARL 4.4.4 and PELMO v5.3.3 were used to predict concentrations in groundwater. One metabolites of mesotrione, MNBA and AMBA were was the major components of the residue considered in groundwater. Due to the pH dependence of degradation and adsorption of mesotrione and the pH dependence of adsorption of AMBA, FOCUS groundwater simulations were run with different combinations of parameters. Worst case sorption endpoints for parent compound and AMBA were run with longest and shortest DT50 for parent compound. The models were also run with specific sorption and degradation values for acid (pH5.1) and alkaline (pH 7.9) soils, to represent 90th and 10th percentile soil pH of maize crop growing areas in Europe. Additionally simulations were run with values for intermediate (pH 6.5). The relationship between degradation and pH for mesotrione was plotted as a linear (non-log) fit and the DT50 values derived from that used as modelling inputs. The relationship between sorption and pH for mesotrione and AMBA were plotted as exponential (log) fits and the Kfoc values derived from that used as modelling inputs. The shortest normalised lab soil DT50 ref value of 4 days for mesotrione was also run with the lowest Kfoc values for mesotrione and AMBA.
The combination of worst case sorption and longest DT50 for parent will not occur in practice as with increasing soil pH mobility and rate of degradation increase. Therefore, results from the set of parameters are considered unrealistically conservative and have not been assessed further.
The applicant has stated that both MNBA were not herbicidally active. To support this statement, results from glasshouse preliminary screening studies were submitted. In these studies, MNBA was applied both pre- and post emergence at doses up to 4000 g a.s./ha, to a range of monocotyledonous and dicotyledonous weed and crop species. None of the test species exhibited any signs of crop damage throughout the studies. This confirms that at doses far in excess of those likely to enter groundwater, MNBA poses no risk to either crops or other vegetation.
Neither metabolite poses any risk to aquatic organisms or to birds and mammals.

With regard to toxicological relevance of MNBA, studies submitted on this metabolite indicates that it is of comparatively low acute toxicity. MNBA is a potential skin sensitiser.
Following EU expert discussion (PRAPeR 134) proposed classification for mesotrione as Reproduction Cat 2 for developmental effects. Based on this proposal, all groundwater metabolites present at >0.1µg/L are considered as relevant unless it can be demonstrated that they would not produce the effects initiating the classification.

The RMS considers the developmental effects to be related to marked disturbances of tyrosine metabolism. The tyrosine disturbance is secondary to inhibition of p-hydroxyphenylpyruvate dioxygenase (HPPD). In a study of relative potency of HPPD inhibition, MNBA was several orders of magnitude less potent than mesotrione. It is considered that MNBA will not produce disturbance of tyrosine metabolism of sufficient magnitude to induce classifiable developmental effects.
It is concluded that MNBA at 0.121 µg/L, in a single scenario, is not a relevant groundwater metabolite of mesotrione.

### 10.8.1 STEP 1: Exclusion of degradation products of no concern

**Not applicable-please refer to point 10.12**

### 10.8.2 STEP 2: Quantification of potential groundwater contamination

**Not applicable-please refer to point 10.12**

### 10.8.3 STEP 3: Hazard assessment – identification of relevant metabolites

### 10.8.3.1 STEP 3, Stage 1: screening for biological activity

**Not applicable-please refer to point 10.12**

### 10.8.3.2 STEP 3, Stage 2: screening for genotoxicity

**Not applicable-please refer to point 10.12**

### 10.8.3.3 STEP 3, Stage 3: screening for toxicity

**Not applicable-please refer to point 10.12**

### 10.8.4 STEP 4: Exposure assessment – threshold of concern approach

**Not applicable-please refer to point 10.12**

### 10.8.5 STEP 5: Refined risk assessment

**Not applicable-please refer to point 10.12**

## 10.9 Relevance assessment of AMBA

# 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<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study<br>Y/N | Owner |
|---|---|---|---|---|---|
| | | | | | |

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

**Cross reference to the section B6 of the dRR**

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<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study<br>Y/N | Owner |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |

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

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

# Appendix 2 Additional information

| | |
|---|---|
| Comments of zRMS: | |