TECHNICAL REPORT

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Outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for mesotrione in light of confirmatory data

European Food Safety Authority (EFSA)

Abstract

The European Food Safety Authority (EFSA) was asked by the European Commission to provide scientific assistance with respect to the risk assessment for an active substance in light of confirmatory data requested following approval in accordance with Article 6(1) of Directive 91/414/EEC and Article 6(f) of Regulation (EC) No 1107/2009. In this context EFSA's scientific views on the specific points raised during the commenting phase conducted with Member States, the applicant and EFSA on the confirmatory data and their use in the risk assessment for mesotrione are presented. The current report summarises the outcome of the consultation process organised by the rapporteur Member State the United Kingdom and presents EFSA's scientific views and conclusions on the individual comments received.

Keywords: mesotrione, peer review, confirmatory data, risk assessment, pesticide, herbicide

Requestor: European Commission

Question number: EFSA-Q-2018-00900

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Summary

Mesotrione was first included in Annex I to Directive 91/414/EEC on 11 July 2003 by Commission Directive 2003/68/EC. Its approval was renewed in accordance with Regulation (EC) No 1107/2009 on 1 June 2017, by Commission Implementing Regulation (EU) No 2017/725, amending Commission Implementing Regulation (EU) No 540/2011. It was a specific provision of the renewal of approval that the applicant was required to submit to the European Commission further studies on:

1. the genotoxic profile of the metabolite AMBA;
2. the potential endocrine disrupting mode of action of the active substance in particular level 2 and 3 tests, currently indicated in the OECD Conceptual framework (OECD 2012) and analysed in the EFSA Scientific opinion on the hazard assessment of endocrine disruptors;
3. the effect of water treatment processes on the nature of residues present in surface and groundwater, when surface water or groundwater are abstracted for drinking water.

The applicant shall submit the relevant information requested under point 1) by 1 July 2017 and the relevant information requested under point 2) by 31 December 2017. The applicant shall also submit the confirmatory information requested under point 3) within a period of two years after a guidance document on evaluation of the effect of water treatment processes on the nature of residues present in surface and groundwater be made public by the Commission.

In accordance with the specific provision, the applicant, Syngenta, submitted an updated dossier to address the confirmatory data requirement 1) on 15 June 2017, and to address the confirmatory data requirement 2) on 21 December 2017. The updated dossier was evaluated by the designated rapporteur Member State (RMS), the United Kingdom, in the form of an addendum to the draft renewal assessment report. In compliance with guidance document SANCO 5634/2009-rev.6.1, the RMS distributed the addendum to Member States, the applicant and EFSA for comments on 18 July 2018. The RMS collated all comments in the format of a reporting table, which was submitted to EFSA on 12 November 2018. EFSA added its scientific views on the specific points raised during the commenting phase in column 4 of the reporting table.

The current report summarises the outcome of the consultation process organised by the RMS, the United Kingdom, and presents EFSA's scientific views and conclusions on the individual comments received.

Mesotrione is the ISO common name for 2-(4-mesyl-2-nitrobenzoyl)cyclohexane-1,3-dione (IUPAC). The representative formulated product for the evaluation was 'Callisto 100 SC' (A12739A), a suspension concentrate (SC) containing 100 g/L mesotrione. The representative use evaluated was application by spraying against annual broadleaved weeds and annual grass weeds in maize.

There was no overall consensus within the peer review to conclude on the endocrine disrupting properties of mesotrione, although there was a general agreement with the RMS assessment that the testis and epididymides findings reported in the multigeneration study should be considered unrelated to mesotrione administration. It is therefore proposed to further discuss the endocrine disrupting properties of the active substance in an experts' consultation. It was agreed that the metabolite AMBA is unlikely to be genotoxic, however it is proposed to further discuss its toxicological profile in an experts' consultation since the metabolite is relevant to consumer exposure.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Mesotrione was first included in Annex I to Directive 91/414/EEC[1] on 11 July 2003 by Commission Directive 2003/68/EC[2]. Its approval was renewed in accordance with Regulation (EC) No 1107/2009[3] on 1 June 2017, by Commission Implementing Regulation (EU) No 2017/725[4], amending Commission Implementing Regulation (EU) No 540/2011[5]. EFSA previously finalised a Conclusion on this active substance on 7 March 2016 (EFSA, 2016).

It was a specific provision of the approval that the applicant was required to submit to the European Commission further studies on:

1. the genotoxic profile of the metabolite AMBA;
2. the potential endocrine disrupting mode of action of the active substance in particular level 2 and 3 tests, currently indicated in the OECD Conceptual framework (OECD 2012) and analysed in the EFSA Scientific opinion on the hazard assessment of endocrine disruptors;
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The applicant shall submit the relevant information requested under point 1) by 1 July 2017 and the relevant information requested under point 2) by 31 December 2017. The applicant shall also submit the confirmatory information requested under point 3) within a period of two years after a guidance document on evaluation of the effect of water treatment processes on the nature of residues present in surface and groundwater be made public by the Commission.

In accordance with the specific provision, the applicant, Syngenta, submitted an updated dossier to address the confirmatory data requirement 1) on 15 June 2017, and to address the confirmatory data requirement 2) on 21 December 2017. The updated dossier was evaluated by the designated rapporteur Member State (RMS), the United Kingdom, in the form of an addendum to the draft assessment report (United Kingdom, 2018). In compliance with guidance document SANCO 5634/2009-rev.6.1 (European Commission, 2013), the RMS distributed the addendum to Member States, the applicant and the EFSA for comments on 18 July 2018. The RMS collated all comments in the format of a reporting table, which was submitted to EFSA on 12 November 2018. EFSA added its scientific views on the specific points raised during the commenting phase in column 4 of the reporting table.

The current report summarises the outcome of the consultation process organised by the RMS, the United Kingdom, and presents EFSA's scientific views and conclusions on the individual comments received.

1.2. Interpretation of the Terms of Reference

On 22 December 2014 the European Commission requested EFSA to provide scientific assistance with respect to the risk assessment of confirmatory data following approval of an active substance in accordance with Article 6(1) of Directive 91/414/EEC and Article 6(f) of Regulation (EC) No 1107/2009. EFSA's scientific views on the specific points raised during the commenting phase

[1] Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. OJ L 230, 19.08.1991, p.1-32.

[2] Commission Directive 2003/68/EC of 11 July 2003 amending Council Directive 91/414/EEC to include trifloxystrobin, carfentrazone-ethyl, mesotrione, fenamidone and isoxaflutole as active substances. OJ L 177, 16.7.2003, p. 12–16

[3] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1-50.

[4] Commission Implementing Regulation (EU) No 2017/725 of 24 April 2017 renewing the approval of the active substance mesotrione in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. OJ L 107, 25.4.2017, p. 24–28

[5] Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. OJ L 153, 11.6.2011, p.1-186.

conducted with Member States, the applicant and EFSA on the risk assessment of confirmatory data for mesotrione are presented.

To this end, a technical report containing the finalised reporting table is being prepared by EFSA. The deadline for providing the finalised report is 10 December 2018.

On the basis of the reporting table, the European Commission may decide to further consult EFSA to conduct a full or focused peer review and to provide its conclusions on certain specific points.

2. Assessment

The comments received on the pesticide risk assessment for the active substance mesotrione in light of confirmatory data and the conclusions drawn by EFSA are presented in the format of a reporting table.

The comments received are summarised in column 2 of the reporting table. The RMS' considerations of the comments are provided in column 3, while EFSA's scientific views and conclusions are outlined in column 4 of the table.

The finalised reporting table is provided in Appendix A of this report.

Documentation provided to EFSA

1. United Kingdom, 2018. Addendum to the assessment report on mesotrione, confirmatory data, July 2018, updated in November 2018. Available online: www.efsa.europa.eu.

2. United Kingdom. Reporting table, comments on the pesticide risk assessment for mesotrione in light of confirmatory data, November 2018.

References

EFSA (European Food Safety Authority), 2016. Conclusion on the peer review of the pesticide risk assessment of the active substance mesotrione. EFSA Journal 2016;14(3):4419, 103 pp. doi: 10.2903/j.efsa.2016.4419

EFSA Scientific Committee, 2011. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 69 pp. doi:10.2903/j.efsa.2011.2379

EFSA Scientific Committee, 2012. Scientific Opinion on Exploring options for providing advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). EFSA Journal 2012;10(7):2750, 103 pp. doi:10.2903/j.efsa.2012.2750

European Commission, 2000. Residues: guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414. SANCO/3029/99-rev. 4, 11 July 2000

European Commission, 2013. Guidance document on the procedures for submission and assessment of confirmatory information following approval of an active substance in accordance with Regulation (EC) No 1107/2009. SANCO 5634/2009-rev. 6.1

OECD (Organisation for Economic Co-operation and Development), 2011. Test No. 456: H295R Steroidogenesis Assay, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264122642-en.

OECD (Organisation for Economic Co-operation and Development), 2012 Guidance document on standardised test guidelines for evaluating chemicals for endocrine disruption. Series on Testing and Assessment. No. 150. ENV/JM/MONO(2012)22.24 August 2012.

OECD (Organisation for Economic Co-operation and Development), 2016. Test No. 476: In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264264809-en.

Abbreviations

AMA	amphibian metamorphosis assay
a.s.	active substance
AUC	area under the curve
DAR	draft assessment report
EATS	Estrogen, androgen, thyroid, steroidogenic
ED	Endocrine disruptor
EU	European Union
HCD	historical control data
HPLC-MS/MS	high-pressure liquid chromatography–mass spectrometry
HPPD	4-Hydroxyphenylpyruvate Dioxygenase
LAGDA	larval amphibian growth and development assay (OECD TG 241
MoA	Mode of action
MRL	maximum residue level
MS	Member State
NOAEL	No observed adverse effect level
NOEL	No observed effect level
PRAPeR	Pesticide Risk Assessment and Peer Review
PRIMo	Pesticide Residue Intake Model
RAR	Renewal assessment report
RMS	rapporteur Member State
TBG	Thyroglobulin
TMDI	theoretical maximum daily intake
ToxCast™	Toxicity ForeCaster
TPO	thyroid peroxidase
TR	thyroid hormone receptor
TTC	Threshold of toxicological concern
US EPA	United States Environmental Protection Agency
WoE	weight of evidence

Appendix A – Collation of comments from Member States, applicant and EFSA on the pesticide risk assessment for the active substance mesotrione in light of confirmatory data and the conclusions drawn by EFSA on the specific points raised

1. Physical/Chemical Properties; Data on application and efficacy; Further Information; Methods of Analysis

Methods of analysis				
No.	**Column 1** **Reference to addendum to assessment report**	**Column 2** **Comments from Member States / applicant / EFSA**	**Column 3** **Evaluation by rapporteur Member State**	**Column 4** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the RMS's assessment of confirmatory data**
1(1)	Vol.3 B.5.1.2.2.6 Method for determination of AMBA in body fluids, p.29	EFSA: the method is validated according to SANCO/3029/99 Rev. 4 for the determination of AMBA in body fluids with a LOQ of 10 ng/mL in plasma and of 30 ng/mL in blood.	UK RMS: Noted, thank you.	Addressed: determination of AMBA in body fluids can be done with LC-MS/MS method with a LOQ of 10 ng/mL in plasma and of 30 ng/mL in blood.

2. Effects on human and animal health

Further toxicological studies				
No.	**Column 1** **Reference to addendum to assessment report**	**Column 2** **Comments from Member States / applicant / EFSA**	**Column 3** **Evaluation by rapporteur Member State**	**Column 4** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the RMS's assessment of confirmatory data**
2(1)	Vol. 3CA, B.6.8.3, Studies on endocrine disruption	EFSA: The reasoning for considering the testis and epididymides findings unrelated to the a.s. administration are agreed. Regarding the thyroid effects, the higher sensitivity of the rat with regards to other species is acknowledged as well as the high	SYN: An environmental assessment taking into consideration the recently published Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 has not been submitted since the guidance was only published on the 7th June	Addressed: The peer review agreed with the RMS assessment that the testis and epididymides findings are unrelated to mesotrione administration. Regarding thyroid findings, see comments 2(3) and 2(9)

Further toxicological studies

No.	Column 1 Reference to addendum to assessment report	Column 2 Comments from Member States / applicant / EFSA	Column 3 Evaluation by rapporteur Member State	Column 4 EFSA's scientific views on the specific points raised in the commenting phase conducted on the RMS's assessment of confirmatory data
		dose at which the increased incidence of thyroid follicular cell adenomas occurred in rats. It is however less clear to which tested species humans' responses to mesotrione exposure should be compared to. Overall we would agree that the arguments given are sufficient to conclude that mesotrione would not present an endocrine disrupting potential regarding EATS modalities relevant to human health. It is noted that the environmental assessment of the endocrine disrupting properties of the a.s. has not been conducted and the assessment has not taken into consideration the recently published Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012[6] and (EC) No 1107/2009.	2018. EFSA's website states that '*The guidance will be used for the assessment of biocides from today (7 June). For pesticides, it will be used in the assessments of those substances for which a decision is scheduled on or after 10 November 2018. This is because the criteria for identifying endocrine disruptors in pesticides were agreed later than those for biocides*.' RMS: Noted, thank you. This assessment was carried out prior to the publication of the guidance document on endocrine disruption. The environmental assessment of ED in the RAR was carried out in accordance with the procedure at the time it was evaluated. The environmental assessment of ED will need to be addressed at renewal.	It is acknowledged that the confirmatory data did not include an ED assessment for mammals and non-target organisms and further consideration would be needed to exclude endocrine disrupting properties for these organisms.
2(2)	B.6.8.3 Studies on endocrine disruption, pp 168	AT: We agree that testes and epididymis weight changes can be considered incidental. For the	SYN: The impact of the new Guidance for the identification of endocrine disruptors in the context of	See comments 2(3) and 2(9)

[6] Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. OJ L 167, 27.6.2012, p. 1–123.

Further toxicological studies				
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		thyroid disruption there is a clear endocrine MoA. Since thyroid effects were only observed in rats and the rat is known to be significantly more sensitive to mesotrione induced tyrosinaemia than humans, thyroid disruption is not relevant for human health assessment. It is noted that for the next renewal of the a.s. approval, it should be considered that ED properties cannot be excluded with regard to the environment based on the available data. The thyroid effects in rats can be considered secondary, however not un-specific. Furthermore, the three available thyroid related *in vitro* assays, conducted as part of the ToxCast™ program, measure only TR-binding, which is not relevant as early key event in the described MoA (disruption occurs at the level of hormone synthesis). Therefore, in order to clarify the environmental ED potential, an AMA or LAGDA could be proposed, and/or a	Regulations (EU) No 528/2012 and (EC) No 1107/2009 (2018) needs to be assessed now that it has been finalised, and the need for further work to investigate the environmental risk will be considered. At the moment the environmental risk to wild mammals is assessed based on a highly conservative NOEL value from a continuous exposure 2-generation rat study, where apical effects on reproduction, growth and development have been taken into consideration in the setting of the NOEL. The rat is the most sensitive mammalian species as demonstrated in the tox database, and it is >40 orders of magnitude more sensitive than the other vertebrates considered in the environmental risk section (birds, fish); it is therefore is believed to be a conservative species to use for wild mammal assessment RMS (human health): Noted, thank you.	

Further toxicological studies

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		comparative study of mesotrione-induced tyrosinaemia across different non-target organism species. However, with regard to human health, mesotrione is not considered to be an ED.		
2(3)	Vol. 3, B.6.8.3 Studies on Endocrine Disruption p. 168	DE: According to Annex I of Reg. 2017/725, the applicant shall provide level 2 and 3 tests (as per the OECD Conceptual Framework) to investigate the potential endocrine disrupting mode of action of mesotrione. Only level 2 studies have been provided. The argument is made that sufficient evidence currently exists to negate CF level 3 tests. It must be noted, however, that a lack of direct receptor binding does not mean that there is no potential for endocrine disruption. Indeed, it is known and has been stated in the report that the mode of action of mesotrione is via interference with the biosynthesis of the hormones, not by direct receptor binding.	RMS: the applicant did not submit any level 3 studies. In the event that level 3 studies were available with some positive results, this would trigger higher tier (level 4 and 5) studies. Therefore there is no reason to perform the level 3 studies. The RMS remains of the opinion that these are not necessary given that the higher tier studies are available.	An experts' consultation is proposed on this point: MS experts to discuss the need for further studies (e.g. level 3 tests, hormone levels) to conclude on the endocrine-disrupting properties of mesotrione. See also comments 2(1), 2(2), 2(4), 2(9)
2(4)	Vol. 3, B.6.8.3 Studies	DE: Were thyroid hormone levels	SYN: Thyroid hormones levels were	See comment 2(3)

Further toxicological studies

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	on Endocrine Disruption p. 171	measured in any of the studies? If so, please report these, too, whether significant differences were seen or not. Similarly, were any effects seen in the pituitary gland histopathology, if assessed?	not measured in the mesotrione studies submitted for regulatory review as such measurements were not required in the relevant guidelines at the time the studies were conducted. Pituitary histopathology was assessed in subchronic and chronic/carcinogenicity studies in both rat and mouse and no mesotrione induced effects were reported. RMS: see applicant response above.	
2(5)	Vol. 3, B.6.8.3 Studies on Endocrine Disruption p. 171	DE: When referring to the elevated TSH levels and their effects on proliferative lesions in the thyroid (Dellarco et al., 2006), it is important to state clearly that while the downstream effects on the thyroid gland are similar, the mechanism of disruption of thyroid hormones is different, namely that thiazopyr induces glucoronidation and thus excretion of T4, whereas mesotrione prevents the synthesis of T3.	RMS: Noted. We agree that there is no evidence that mesotrione leads to enhanced metabolism/excretion of the thyroid hormones. The RAR has been amended with some further explanation. However, this does not compromise the final conclusion that mesotrione does not have a direct effect on the endocrine system.	Addressed: Additional clarifications have been added to the revised RAR.
2(6)	Vol. 3, B.6.8.3 Studies	DE: In the new WoE analysis, there is	SYN: This study examined the	Addressed:

Further toxicological studies				
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	on Endocrine Disruption p. 173	only one sentence stating that according to the ToxCast analysis "The authors found no evidence that mesotrione has any ability to influence the biosynthesis of any of the nine steroid hormones evaluated in this assay." As a minimum, this statement needs to be elaborated on with more details about what parameters were examined and how interference with biosynthesis was determined.	synthesis of nine steroid hormones (deoxycorticosterone, pregnenolone, progesterone, androstenedione, cortisol, deoxycortisol, oestradiol, progesterone, testosterone) in H295R cells in response to 48 hour treatment with mesotrione. Steroid hormones were quantified using HPLC-MS/MS. Increases and decreases in steroidogenic activity were determined through comparison to concurrent controls with a threshold of a 1.5 fold change or greater being used to determine active compounds. This protocol was developed by the US EPA and is similar to the H295R study methodology adopted by the OECD in test guideline 456 (OECD, 2011). RMS: See response from applicant above. This explanation has been added to the RAR. See also comments 2(1), 2(2) and 2(8).	Additional details have been provided in the revised RAR.
2(7)	Vol. 3, B.6.8.1.3 Genotoxicity p. 138	DE: While the clastogenic potential or lack thereof has now been resolved, the mutagenic potential of AMBA in mammalian has not been adequately tested. An *in*	RMS: According to the EFSA Scientific Opinion on genotoxicity testing strategies (EFSA Scientific Committee, 2011)) a mammalian gene mutation assay is not routinely required.	Addressed.

Further toxicological studies

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		vitro mammalian cell mutation assay performed according to OECD TG 476 (OECD, 2016) is still required to clarify this point.	Although the scientific opinion that an in micronucleus(MN) assay should be performed, the presence of a well performed in vivo MN assay is considered to meet, if not exceed, this requirement. The RMS considers the genotoxicity of AMBA has been adequately addressed and no further genotoxicity testing is necessary. See also comments 2(10), 2(11) and 2(13).	
2(8)	B.6.8.3 Studies on endocrine disruption	FR: While an assessment of ED potential according to the new EU guidance for identification of endocrine disruptors would have been valuable, the conclusion regarding effects on testis and epididymis could be agreed upon based on the provided HCD as well as ToxCast™ data.	Noted, thank you	See comment 2(1)
2(9)	B.6.8.3 Studies on endocrine disruption	FR: As regard thyroid effects observed in top dose rats in the 2-year study (hyperplasia in both males and females and increased incidence of adenoma in females), they are considered secondary to the severe tyrosinaemia: free	SYN: Effects on follicular cells in the thyroid have been considered as a continuum: hypertrophy – neoplasia. If the sum of effects is considered then both males and females are affected, the total number of lesions noted in males being higher than that in	An experts' consultation is proposed on this point: MS to discuss the endocrine-disrupting potential of mesotrione. See also 2(1), 2(2), 2(3)

Further toxicological studies

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		tyrosine acting as a competitive inhibitor of the TBG-iodination (iodine organification) activity of TPO and free iodotyrosines inhibiting the active uptake of iodide by the thyroid. However, no specific mechanistic data have been carried out to substantiate the postulated mode of action through tyrosinaemia and to exclude other possible modes of action. Furthermore, the more severe effects observed in female rats (thyroid adenoma) compared to males challenge the hypothesis since tyrosinaemia was more critical in male.	females, supporting the hypothesis that thyroid effects are attributable to severe tyrosinaemia. RMS: Noted. We agree there is an absence of direct investigation to support the MOA on thyroid (eg measurement of thyroid hormone levels). The proposed MOA is considered plausible. See also comments 2(1) and 2(2).	

Dose level of mesotrione (ppm)	Males				Females			
	0	7.5	100	2500	0	7.5	100	2500
Lesion Type								
Follicular cyst	1	0	3	5	0	0	1	0
Diffuse hyperplasia	0	0	0	1	1	0	0	0
Cyst with hyperplasia	1	5	7	5	0	0	1	3
Focal hyperplasia	0	1	0	0	0	0	0	0
Adenoma	0	1	3	1	0	1	1	4
Carcinoma	0	1	0	1	0	0	0	0
Total	**2**	**8**	**13**	**13**	**1**	**1**	**3**	**7**

Toxicological data on metabolites

No.	Column 1 Reference to addendum to assessment report	Column 2 Comments from Member States / applicant / EFSA	Column 3 Evaluation by rapporteur Member State	Column 4 EFSA's scientific views on the specific points raised in the commenting phase conducted on the RMS's assessment of confirmatory data
2(10)	Vol. 3CA, B.6.8.1.3 Genotoxicity - AMBA	EFSA: we agree with the RMS conclusion that the micronucleus test gave sufficient evidence of lack of genotoxic (clastogenic and aneugenic) potential of the metabolite AMBA since bone marrow exposure was demonstrated after 2 dosing with the substance with 24 h interval and measurement of AMBA in whole blood. We agree with the RMS that the confirmatory data requirement (1) has been fulfilled. It is however noted that the data gap identified in the EFSA conclusion (EFSA, 2016) regarding the relative toxicity of the metabolite compared with mesotrione has not been addressed.	SYN: A metabolism study showing that, in rats, orally dosed MNBA is rapidly metabolised to AMBA was submitted as part of ai renewal. Recently, a more detailed study has been completed and demonstrates that orally dosed MNBA is rapidly metabolised in the rat such that comparison of the systemic exposure quantified in terms of blood AUC (0-24) shows a 2-3 fold higher exposure to the AMBA (R44276) compared to MNBA, over the 24 hour period following dosing with MNBA (CA3511).[7] Based on this Syngenta believes that MNBA repeat dose exposure studies can be used to evaluate relative toxicity of both AMBA and MNBA to mesotrione. Based on these studies both MNBA and AMBA are of lower toxicity than mesotrione in the rat. The study report is available upon request.	An experts' consultation is proposed on this point since the additional information provided was not peer reviewed and the metabolite is relevant to consumer exposure: MSs experts to discuss whether the toxicological profile of AMBA may be considered as covered by the toxicological studies performed on MNBA and, if applicable, to establish toxicological reference values. See also 2(14)

[7] CA3511 and R44276- Systemic Exposure of CA3511 and R44276 in the Rat Following Single Oral Administration of CA3511. Pulner M (2017) Report No 38654 (Syngenta File no CA3511-10034)

Toxicological data on metabolites				
No.	**Column 1** **Reference to addendum to assessment report**	**Column 2** **Comments from Member States / applicant / EFSA**	**Column 3** **Evaluation by rapporteur Member State**	**Column 4** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the RMS's assessment of confirmatory data**
			RMS: Although the proposal from the EFSA conclusion regarding the comparative toxicities of mesotrione and AMBA is noted, this was carried through to the Implementing Regulation (2017/725). There is no data on the repeated dose toxicity of AMBA. However, there are 28- and 90-day studies on MNBA and a metabolism study that shows 10% of an administered dose of MNBA is present in the urine as AMBA. The NOAEL for the 28d study with MNBA is 1000 mg/kg bw/d and the NOAEL for the 90-day study is 51 mg/kg bw/d (based on b.wt and food consumption). If all the toxicity exhibited in the 90-day study with MNBA is due to AMBA, this indicates that the NOAEL for AMBA in a 90-day study in rats would be ~5 mg/kg bw/d. There are two 90-day studies with mesotrione. These gave NOAELs of 0.1 and 0.47 mg/kg bw/d. When dose spacing is considered	

Toxicological data on metabolites				
No.	**Column 1** **Reference to addendum to assessment report**	**Column 2** **Comments from Member States / applicant / EFSA**	**Column 3** **Evaluation by rapporteur Member State**	**Column 4** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the RMS's assessment of confirmatory data**
			the most relevant NOAEL is 0.47 mg/kg bw/d. This is approximately 10-fold lower than the extrapolated 90-day NOAEL for AMBA. In addition AMBA and MNBA were found to show only slight inhibition of HPPD at concentrations where mestrione showed complete inhibition of HPPD activity. Therefore the overall WoE is that AMBA is less toxic than mesotrione based on the available data. It should also be noted that the need for a comparison of the toxicity of AMBA and mesotrione should be driven by exposures to AMBA residues. The UK calculations based on the available data using PRIMo Rev. 2, indicate that intakes of AMBA are <10% of the TTC for a Cramer class III substance (1.5 µg/kg bw/d). Use of the TTC is a valid scientific method promoted by the EFSA Scientific Committee (EFSA Scientific Committee, 2012) and therefore the RMS considers that no further assessment of AMBA is	

Toxicological data on metabolites

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			required. The RAR has been amended to include the above consideration.	
2(11)	B.6.8.1.3 Genotoxicity, AMBA, pp 136	AT: We agree with the conclusion of the RMS that overall AMBA has no genotoxic potential based on the results of the test battery.	UK RMS: Noted, thank you.	Addressed.
2(12)	Vol 3, B 6.8.1.6 pg 142, para 2	SYN: The text 'It was agreed at PRAPer 134 that a genotoxic potential in vivo could not be excluded…' (re to AMBA) should be reworded to include the data from the in vivo study reported in B 6.8.1.3 'AMBA is not genotoxic based on the results of the battery of tests undertaken with this metabolite'	RMS: Noted. The text has been amended.	Addressed.
2(13)	B 6.8.1.3 Genotoxicity AMBA micronucleus test	FR: It is agreed that AMBA is neither clastogenic nor aneugenic in the rat bone marrow nucleus assay and proof of exposure of the bone marrow has been properly demonstrated. Therefore, genotoxic potential of AMBA is considered clarified.	RMS: noted thank you.	Addressed.

Toxicological data on metabolites				
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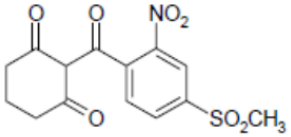
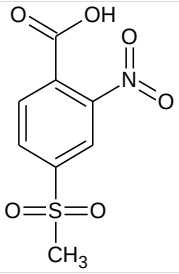
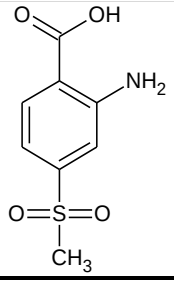
2(14)	B.6.8.General toxicity AMBA	FR: Since AMBA is also a plant metabolite, its toxicological profile compared to mesotrione needs also to be addressed.	SYN: See response to 2(10) RMS: See response to 2(10).	See proposed experts' consultation in 2(10)

Appendix B – Used compound codes

Code/trivial name[a]	Chemical name/SMILES notation[b]	Structural formula[c]
mesotrione	2-(4-mesyl-2-nitrobenzoyl)cyclohexane-1,3-dione KPUREKXXPHOJQT-UHFFFAOYSA-N	
MNBA	4-(methylsulfonyl)-2-nitrobenzoic acid O=S(C)(=O)c1cc(c(cc1)C(=O)O)N(=O)=O	
AMBA	2-amino-4-(methylsulfonyl)benzoic acid O=S(C)(=O)c1cc(N)c(cc1)C(=O)O KFOGGDGNOLZBNY-UHFFFAOYSA-N	

(a): The metabolite name in bold is the name used in the conclusion.
(b): ACD/Name 2017.2.1 ACD/Labs 2017 Release (File version N40E41, Build 96719, 06 Sep 2017)
(c): ACD/ChemSketch 2017.2.1 ACD/Labs 2017 Release (File version C40H41, Build 99535, 14 Feb 2018)