TECHNICAL REPORT

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Outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for iodosulfuron and prosulfuron 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 iodosulfuron and prosulfuron are presented. The current report summarises the outcome of the consultation process organised by the rapporteur Member State Sweden and co-rapporteur Member State France and presents EFSA's scientific views and conclusions on the individual comments received.

Keywords: prosulfuron, iodosulfuron, triazine amine, peer review, confirmatory data, risk assessment, pesticide, metabolite

Requestor: European Commission

Question number: EFSA-Q-2018-00528

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Summary

The approval of iodosulfuron has been renewed on 1 April 2017 by Commission Regulation (EU) No 2017/407 in accordance with Regulation (EC) No 1107/2009 and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. EFSA previously finalised a Conclusion on this active substance on 31 March 2016.

The approval of prosulfuron has been renewed on 1 May 2017 by Commission Regulation (EU) No 2017/375 in accordance with Regulation (EC) No 1107/2009 and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. EFSA previously finalised a Conclusion on this active substance on 18 August 2014.

It was a specific provision of the approval that the applicant was required to submit to the European Commission further information as regards the genotoxic potential of the metabolite triazine amine (CGA150829) to confirm that this metabolite is not genotoxic and not relevant for risk assessment. In accordance with the specific provision, the applicant, the Aminotriazine Task Force (originally Bayer AG, Syngenta AG and DuPont, later expanded to include FMC Corporation), submitted a weight of evidence assessment in December 2017, which was evaluated by the designated rapporteur Member State (RMS) Sweden (RMS for iodosulfuron), and co-RMS France (RMS for prosulfuron) in the form of an addendum to the draft 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 5 April 2018. The RMS collated all comments in the format of a reporting table, which was submitted to EFSA on 27 June 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 Sweden and co-RMS France, and presents EFSA's scientific views and conclusions on the individual comments received.

There was general agreement that triazine amine does not induce gene mutations in bacteria *in vitro* and chromosome aberration *in vitro*. However, no firm conclusion could be drawn regarding the gene mutation potential of triazine amine on the basis of the confirmatory information submitted, since some issues were identified with regard to the quality and the interpretation of the results of two *in vitro* gene mutation studies.

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1. Introduction

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

The approval of iodosulfuron has been renewed on 1 April 2017 by Commission Regulation (EU) No 2017/407[1] in accordance with Regulation (EC) No 1107/2009[2] and amending the Annex to Commission Implementing Regulation (EU) No 540/2011[3]. EFSA previously finalised a Conclusion on this active substance on 31 March 2016 (EFSA, 2016).

The approval of prosulfuron has been renewed on 1 May 2017 by Commission Regulation (EU) No 2017/375[4] in accordance with Regulation (EC) No 1107/2009 and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. EFSA previously finalised a Conclusion on this active substance on 18 August 2014 (EFSA, 2014).

It was a specific provision of the approval that the applicant was required to submit to the European Commission further information as regards the genotoxic potential of the metabolite triazine amine (CGA150829) to confirm that this metabolite is not genotoxic and not relevant for risk assessment. The applicant was requested to submit that information to the Commission, the Member States and the Authority by 1 (Iodosulfuron) and 31 (Prosulfuron) October 2017.

In accordance with the specific provision, the applicant, the Aminotriazine Task Force (originally Bayer AG, Syngenta AG and DuPont, later expanded to include FMC Corporation), submitted a weight of evidence assessment in December 2017, which was evaluated by the designated rapporteur Member State (RMS) Sweden (RMS for iodosulfuron), and co-RMS France (RMS for prosulfuron) in the form of an addendum to the draft assessment report (Sweden, 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 EFSA for comments on 5 April 2018. The RMS collated all comments in the format of a reporting table, which was submitted to EFSA on 27 June 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 Sweden and co-RMS France and presents EFSA's scientific views and conclusions on the individual comments received.

Confirmatory data as regards the genotoxic potential of the metabolite triazine amine was requested for four sulfonylurea substances that have been renewed so far. Confirmatory data were submitted to Slovenia (RMS for metsulfuron-methyl) in September 2016, and to UK (RMS for thifensulfuron-methyl) in March 2017. The outcome of the consultation process on the evaluation done by Slovenia, EFSA's scientific views and conclusions on individual comments received was published by EFSA (EFSA, 2017).

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] Commission Implementing Regulation (EU) 2017/407 of 8 March 2017 renewing the approval of the active substance iodosulfuron 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 63, 9.3.2017, p. 87–90.

[2] 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.

[3] 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.

[4] Commission Implementing Regulation (EU) 2017/375 of 2 March 2017 renewing the approval of the active substance prosulfuron, as a candidate for substitution, 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 58, 4.3.2017, p. 3–7

conducted with Member States, the applicant and EFSA on the risk assessment of confirmatory data for iodosulfuron and prosulfuron 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 25 July 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 metabolite triazine amine in light of confirmatory data and the conclusions drawn by the 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. Sweden, 2018a. Evaluation of confirmatory data for the active substances iodosulfuron and prosulfuron under Regulation (EC) No 1107/2009, Common metabolite to sulfonyl urea active substances: triazine amine, April 2018, updated in June 2018. Available online: www.efsa.europa.eu.

2. Sweden, 2018b. Reporting table, comments on the pesticide risk assessment for prosulfuron and iodosulfuron in light of confirmatory data, June 2018.

References

EFSA (European Food Safety Authority), 2014. Conclusion on the peer review of the pesticide risk assessment of the active substance prosulfuron. EFSA Journal 2014;12(9):3815, 94 pp. https://doi.org/10.2903/j.efsa.2014.3815

EFSA (European Food Safety Authority), 2015a. Conclusion on the peer review of the pesticide risk assessment of the active substance metsulfuron-methyl. EFSA Journal 2015;13(1):3936, 106 pp. doi:10.2903/j.efsa.2015.3936

EFSA (European Food Safety Authority), 2015b. Conclusion on the peer review of the pesticide risk assessment of the active substance thifensulfuron-methyl. EFSA Journal 2015;13(7):4201, 144 pp. doi:10.2903/j.efsa.2015.4201

EFSA (European Food Safety Authority), 2016. Conclusion on the peer review of the pesticide risk assessment of the active substance iodosulfuron-methyl-sodium (approved as iodosulfuron). EFSA Journal 2016;14(4):4453, 111 pp. https://doi.org/10.2903/j.efsa.2016.4453

EFSA (European Food Safety Authority), 2017. Technical report on the outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for metsulfuron-methyl in light of confirmatory data. EFSA supporting publication 2017:EN-1257. 44 pp. doi:10.2903/sp.efsa.2017.EN-1257

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. https://doi.org/10.2903/j.efsa.2011.2379

EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger M, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Silano V, Solecki R, Turck D, Younes M, Aquilina G, Crebelli R, Gurtler R, Hirsch-Ernst KI, Mosesso P, Nielsen E, van Benthem J, Carfi M, Georgiadis N, Maurici D, Parra Morte J and Schlatter J, 2017. Scientific Opinion on the clarification of some

aspects related to genotoxicity assessment. EFSA Journal 2017;15(12):5113, 25 pp. https://doi.org/10.2903/j.efsa.2017.5113

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

Sweden, 2017. Revised Renewal Assessment Report (RAR) on tribenuron-methyl prepared by the rapporteur Member State Sweden in the framework of Regulation (EC) No 1107/2009, May 2017. Available online: www.efsa.europa.eu

Abbreviations

a.s.	active substance
ADME	absorption, distribution, metabolism, excretion
CHO	Chinese hamster ovary cells
DMSO	dimethyl sulfoxide
DNA	DeoxyriboNucleic Acid
GEF	global evaluation factor
HPRT	hypoxanthine phosphorybosyl transferase
LD_{50}	lethal dose, median; dosis letalis media
MF	mutant frequency
MLA	mouse lymphoma assay
QSAR	Quantitative structure–activity relationship
RMS	rapporteur Member State
RTG	relative total growth
TG	test guideline
TF	Task force
TK	thymidine kinase
UDS	unscheduled DNA synthesis
XPRT	xanthine-guanine phosphorybosyl transferase
WoE	weight of evidence

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

Mammalian toxicology

Genotoxicity

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)	General	UK: Many thanks for a clear and thorough evaluation of the genotoxicity WoE analysis of triazine amine.	RMS June 2018: Thank you. Addressed	Addressed
2(2)	3.0	Applicant (Aminotriazine Taskforce (TF)): We acknowledge and appreciate the detailed analysis carried out by the RMS concerning the genotoxicity of triazine amine, the 18 genotoxicity studies, information concerning QSAR and read across, and ADME data as part of the confirmatory data evaluation for Iodosulfuron and Prosulfuron under Regulation (EC) 1107/2009. The RMS has concluded that there are no results supporting that triazine amine induces gene mutations or chromosome aberrations in mammalian cells *in vitro*. As a result, the genotoxicity of triazine amine can be	RMS June 2018: Thank you for support. Addressed Co-RMS June 2018: Noted. Addressed	Addressed

		concluded and no further information, particularly animal studies, is needed. The Aminotriazine Taskforce agrees with this conclusion.		
2(3)	Toxicology and metabolism: Genotoxicity	DE: DE applauds the work by both the RMS and co-RMS in preparing this sorely needed overview concerning the genotoxic potential of IN-A4098. Nonetheless, DE disagrees with the RMS on several key assessments and with the conclusion that the evidence does not irrefutably indicate that IN-A4098 is free of a genotoxic potential. This is based on the findings of the in vitro mammalian cell mutagenicity studies. See specific comments below.	RMS June 2018: Thank you. Regarding conclusions on the *in vitro* mammalian gene mutagenicity studies, see comments 2(19) and 2(28). Addressed Co-RMS June 2018: Thank you for your support. Addressed	Addressed
2(4)	3.0 Applicant's summary of available data	Applicant (Aminotriazine TF): The table contains a summary of the Clarke CHO/HPRT study which states: Negative results in the presence of S9, equivocal increase of gene mutation in the absence of S9. We wish to clarify that our position is that this study is negative both in the absence and presence of S9. The text in the table presents the conclusion by some RMSs and EFSA.	RMS June 2018: We understand, and suggest that we could clarify the applicant's view in Table 3-1 in a revised report. Co-RMS June 2018: Agrees with the RMS. Addressed	It is noted that Co-RMS, DE and EFSA consider the CHO/HPRT study (Clarke 2009) negative in the presence of S9 and equivocal in the absence of S9 (or positive according to DE on the basis of reassessment by applying OECD 2016). On the other hand the RMS, Applicant and UK consider this study negative both in the absence and in the presence of S9. However, the RMS considered the CHO/HPRT study (Clarke 2009) of low quality (because of low number of cells) and it did not accept to consider the results of this study in a WoE assessment.

				Peer review is proposed to discuss how to interpret the findings of the CHO/HPRT study (Clarke 2009) and to discuss whether this study should meet the requirements of an older version or the current revision of an OECD guideline to assess the correct number of cells treated. See also 2(16), 2(17), 2(18), 2(19), 2(20), 2(21) and 2(31)
2(5)	3.1.1, bacterial reverse mutation studies, pag. 7	EFSA: six bacterial reverse mutation studies have been provided and they were all negative. However, a summary of material and methods and main results of such studies is missing, together with a critical assessment by the RMS.	<u>RMS June 2018:</u> It was not considered as part of our task to present material and methods and results of all studies. The task was to evaluate the weight of evidence assessment submitted as confirmatory data ([redacted] 2017). For that purpose it was necessary to check each study carefully in order to judge whether each study was acceptable and whether or not each study should be included in the weight of evidence assessment. This was done and all six bacterial reverse mutation studies were considered acceptable and included in the WoE analysis. Please also note that the studies have previously been presented in assessment reports on different sulfonylurea active substances, and no concerns were raised. Addressed	Addressed

			Co-RMS June 2018: Agrees with the RMS. Addressed	
2(6)	General comment on Ames test	EFSA: triazine amines are common metabolites to different active substances. According to the EFSA assessment provided for thifensulfuron-methyl, chlorsulfuron, triasulfuron, prosulfuron, metsulfuron-methyl, tribenuron-methyl, iodosulfuron-methyl and triflusulfuron the bacterial mutagenicity of triazine amine tested in *in vitro* by mean of the bacterial reverse mutation atest (Ames test) was clearly negative.	RMS June 2018: Thank you for supporting the conclusion that all six bacterial mutation studies were negative. Addressed. Co-RMS June 2018: Thank you for your support. Addressed	Addressed
2(7)	3.1.1 and 4.3.1 – Ames studies	UK: We agree with RMS and co-RMS that the six available Ames tests are negative and that **triazine amine is not mutagenic in bacteria**.	RMS June 2018: Thank you for supporting the conclusion that triazine amine does not induce gene mutations in bacteria. Addressed. Co-RMS June 2018: Thank you for your support. Addressed.	Addressed
2(8)	3.1.1 Bacterial reverse mutation studies	Applicant (Aminotriazine TF): We agree with the RMS and co-RMS that all six bacterial mutation studies are negative.	RMS June 2018: Thank you for supporting the conclusion that all six bacterial mutation studies were negative. Addressed.	Addressed

			Co-RMS June 2018: Thank you for your support. Addressed.	
2(9)	Toxicology and metabolism: Genotoxicity	DE: DE agrees that all bacterial mutagenicity studies were valid and none demonstrated mutagenic potential of IN-A4098 in bacteria. DE considers the mutagenic potential of IN-A4098 in bacteria to have been sufficiently investigated.	RMS June 2018: Thank you for supporting the conclusion that none of the bacterial mutagenicity studies demonstrated a mutagenic potential. Addressed. Co-RMS June 2018: Thank you for your support. Addressed.	Addressed
2(10)	3.1.2, *in vitro* mammalian chromosome aberration studies, study by Dollenmeier 1987, pag. 8	EFSA: it is recognised that the study was not performed according to OECD TG 473 and therefore that no relevant conclusion can be drawn. The most important arguments to dismiss this study have been presented, but it would be appreciated if a summary of materials and methods together with the main results are better detailed.	RMS June 2018: We do not find it relevant to present material and methods and results for a study which clearly deviated from test guidelines and which therefore can be considered as not acceptable and not useful in the weight of evidence assessment. Addressed. Co-RMS June 2018: Agrees with the RMS. Addressed.	Noted
2(11)	General comment on *in vitro* mammalian chromosome aberration test	EFSA: in addition to the studies presented in this dossier, an additional study (an *in vitro* chromosome aberration test in human lymphocytes) has been provided by Gudi et al., 2009 for IN-A4098, a metabolite also of	RMS June 2018: Thank you for the information. The results of the study by Gudi et al. (2009) further strengthen the evidence that triazine amine does not induce chromosome aberrations in mammalian cells *in vitro*.	EFSA noted that an additional study (an *in vitro* chromosome aberration test in human lymphocytes by Gudi et al., 2009) has been provided for IN-A4098 in the RAR on tribenuron (Sweden, 2017). The results of such study were clearly negative and further

		tribenuron, recently assessed by EFSA. The results were clearly negative.	Open point for the RMS to indicate (in section 4.3.2) that during peer review EFSA informed that an additional *in vitro* study has been made available to EFSA (Gudi et al, 2009) which further supports the conclusion that triazine amine does not induce chromosome aberrations in mammalian cells *in vitro*. Co-RMS June 2018: Agrees with the RMS. Addressed.	supports the conclusion that triazine amine does not induce chromosome aberrations in mammalian cells *in vitro*.
2(12)	3.1.2 *In vitro* mammalian chromosome aberration studies	Applicant (Aminotriazine TF): We agree with the RMS and co-RMS that the Dollenmeier (1987) study is of poor quality, that the results are unreliable and that therefore the study should be excluded in the WoE evaluation concerning the potential clastogenicity of triazine amine. We also agree that the four remaining *in vitro* chromosome aberration studies – Meyer (1991), Roy and Rao (2009), Flügge (2011), and Woods (2011) – are negative and confirm that triazine amine is not clastogenic.	RMS June 2018: Thank you for your support. Addressed. Co-RMS June 2018: Thank you for your support. Addressed.	Addressed
2(13)	3.1.2 and 4.3.2 – IVC studies in mammalian cells	UK: We agree with the RMS that three (Roy and Rao, 2009; Flugge, 2011c; Woods, 2011a) of the five available studies are reliable and should be included in the WoE evaluation. These were all	RMS June 2018: Thank you for supporting the conclusion that triazine amine does not induce chromosome aberrations in mammalian cells *in vitro*. Addressed.	Addressed

		negative valid studies. Therefore, we agree with the RMS that **triazine amine is not clastogenic *in vitro* in mammalian cells**. Roy and Rao (2009) and Woods (2011a) were also submitted to the UK in the context of the confirmatory data requirements for thifensulfuron-methyl. We agree that the small deviations from OECD TG 473 identified do not compromise the validity of these studies. In particular, we agree that the new requirement for the scoring of 300 metaphases (rather than 200) introduced by the 2016 revision of the guideline should not be applied when considering the validity of such studies, which were conducted several years before such revision of the guideline.	Co-RMS June 2018: Thank you for your support. Addressed.	
2(14)	Conclusion on gene mutation in bacteria and chromosome aberrations	AT: AT agrees with RMS and Co-RMS that there is no concern for gene mutation in bacteria and chromosome damage as endpoint.	RMS June 2018: Thank you for supporting the conclusion that triazine amine does not induce gene mutations in bacteria or chromosome aberrations in mammalian cells *in vitro*. Addressed. Co-RMS June 2018: Thank you for your support. Addressed.	Addressed
2(15)	3.1.3, *in vitro*	EFSA: it would be appreciated that the	RMS June 2018:	Addressed

	mammalian gene mutation studies, pag. 10	*in vitro* mammalian gene mutation studies in Chinese hamster ovary cells are presented immediately after the bacterial reverse mutation studies, as following also the order indicated in Table 3-1.	In sections 3.1.1-3.1.5 we presented the different studies in the order different studies are mentioned in the data requirements. We suggest that the order in which the separate studies are presented in Table 3-1 could be modified in a revised report to correspond to the order of presentation in section 3.1.1 - 3.1.5. Open point for the RMS to modify the order in which studies are presented in Table 3-1 (to correspond with sections 3.1.1-3.1.5). Co-RMS June 2018: Agrees with the RMS. Addressed.	
2(16)	3.1.3 and 4.3.3 – *In vitro* MCGM studies – Clarke, 2009	UK: We agree with the conclusion of the RMS that overall **triazine amine is not mutagenic *in vitro* in mammalian cells**. However, we reach the same conclusion by a different interpretation of the data (Clarke, 2009; Woods, 2011b and Lloyd, 2016a) submitted to the UK in the context of the confirmatory data requirements for thifensulfuron-methyl. A re-evaluation of the Clarke (2009) CHO/HPRT study (originally considered equivocal) shows that it is negative as there are no statistically significant differences in mutant frequencies	RMS June 2018: Thank you for supporting the conclusion that triazine amine does not induce gene mutations in mammalian cells *in vitro* (but see also 2(27)). Regarding the comment on whether a study should meet the requirements of an older version or the current revision of an OECD guideline where progress in mutagenicity testing improving the ability to distinguish a mutagen from a non-mutagen has resulted in modifications of the requirements, we are of the opinion that priority should be given to current revised versions. In the case commented here the issue is the required number of cells that	It is noted that RMS considers the CHO/HPRT study (Clarke 2009) of low quality as the number of cell treated was lower than that required by the current revision of the OECD guideline (476, 2016). On the other hand, Applicant, UK, SI and Co-RMS disagree and consider this study can be used in the weight of evidence. Co-RMS, DE, SI, AT and EFSA do not agree with the conclusions proposed for this study, which should rather be considered as equivocal due to the biological relevance of the concentration-related increased mutant frequency observed at the two highest dose levels without metabolic activation.

		between controls and treated cultures, no statistically significant linear trends in dose response and the mutant frequencies in treated cultures are below the threshold of $40x10^6$. The UK disagrees with the RMS that, as the number of cell treated was lower than that required by the current revision of the OECD guideline (476, 2016), the study is not reliable. We consider that such a new requirement, introduced by the 2016 revision of the guideline, should not be applied when considering the validity of the study, which was conducted several years before the revision of the guideline. Therefore, we consider this to be a sufficiently reliable negative study, which contributes to the overall WoE. Clearly, the limitation identified by the RMS does not make the study completely unreliable. As a minimum, it should be considered supportive/supplementary of the other negative studies.	should be tested, which is of obvious importance for detecting an effect if it exists. It is commented by UK that in this case it would be sufficient to comply with the requirements of the 1997 version of OECD 476. We find that, even according to the 1997 version of the guideline, the number of cells used were not sufficiently high. This is discussed below. OECD 476 (v1997) states (in §8): "*The minimal number of viable cells surviving treatment and used at each stage in the test should be based on the spontaneous mutation frequency. A general guide is to use a cell number which is at least ten times the inverse of the spontaneous mutation frequency. However, it is recommended to utilise at least 10^6 cells.*" In the present experiment without S9 the spontaneous mutation frequency (solvent control) was $6.1/10^6$ viable cells. Ten times the inverse of this frequency is $1.64x10^6$ (10x(1/0.0000061)), which is the number of viable cells that should have been used for mutant selection in each experimental group. Clarke (2009) reported that ten plates, each with $2x10^5$ cells, were used in each experimental group for mutant selection (i.e. in total $2x10^6$ cells). In all treated groups except the 100 µl/mL group the viability of cells was	See peer review proposed in 2(4) See also 2(17), 2(18), 2(19), 2(20), 2(21) and 2(31)

			around 70%, meaning that about $1.4x10^6$ viable cells were used for mutant selection, which is below the guideline recommendation of $1.64x10^6$ viable cells. In the 100 µl/mL group the viability of cells was 81%, meaning that $1.62x10^6$ viable cells were used for mutant selection, i.e. almost reaching the guideline recommendation of $1.64x10^6$. Note that the guideline also states that at least 10^6 cells should be used. This is applicable for cases where high spontaneous mutant frequencies would result in calculated numbers of viable cells below 10^6 that should be used for mutant selection. This is not the case here. In any case, the RMS propose that from a scientific viewpoint it is more relevant to compare the study with the updated version (2016) of the guideline. OECD 476 (v2016) states (in §26): *"The minimum number of cells used for each test (control and treated) culture at each stage in the test should be based on the spontaneous mutant frequency. A general guide is to treat and passage sufficient cells as to maintain 10 spontaneous mutants in every culture in all phases of the test (17). The spontaneous mutant frequency is generally between 5 and 20 $x10^{-6}$. For a spontaneous mutant frequency of 5 $x10^{-6}$ and to maintain a sufficient number of spontaneous mutants (10*

			or more) even for the cultures treated at concentrations that cause 90% cytotoxicity during treatment (10% RS), it would be necessary to treat at least 20 x 10^6 cells. In addition a sufficient number of cells (but never less than 2 million) must be cultured during the expression period and plated for mutant selection (17)." The low number of cells used reduced the statistical power of the test. The low power of the test also means that the negative result observed has low reliability. Accordingly, due to the unacceptable quality we think that the study should not be included in the weight of evidence analysis of potential mutagenicity of triazine amine. Addressed. <u>Co-RMS June 2018:</u> We agree with UK that the Clarke study should be considered as acceptable and the results as reliable. Nevertheless, we do not agree with the conclusions proposed for this study, which should rather be considered as equivocal due to the biological relevance of the concentration-related increased mutant frequency observed at the two highest dose levels without metabolic activation. Addressed.	

2(17)	3.1.3 *In vitro* mammalian gene mutation studies	Applicant (Aminotriazine TF): We agree with the RMS that the slightly greater mutant frequencies observed in the CHO/HPRT study by Clarke (2009) were likely due to chance given the large variation in background mutants in this assay. Hence, as general practice at the time this study was conducted, the value of 50 mutants/10^6 cells was the threshold for considering a substance to have potentially induced gene mutations (Li et al., 1988). However, the testing laboratory used a more conservative minimum of >40 mutants/10^6 cells. None of the mutant frequencies exceeded this value, none of the mutant frequencies were statistically significant relative to control, nor was there a statistically significant trend.	<u>RMS June 2018:</u> The main conclusion by the RMS is that the negative result of the study should not be used in a WoE assessment due to the low statistical power of the test (insufficient number of cells used, see response to comment 2(16)). Regarding the use of threshold values for determining a positive (or negative) result, the RMS would like to draw attention to the fact that neither in OECD 476 (v2016) nor in OECD 476 (v1997), the use of a threshold value is not indicated as an option for evaluation and interpretation of results. Instead, any increase in mutant frequency which is statistically significant (and biologically relevant) would be sufficient to demonstrate a positive result. See also 2(20). Addressed. <u>Co-RMS June 2018:</u> We considered the study by Clarke as equivocal as the biological relevance of the concentration-related increased mutant frequency observed at the two highest dose levels without metabolic activation cannot be excluded. According to OECD 476 (1997) in place at the time the study was conducted: "*There are several criteria for determining a positive result, such as a concentration- related, or a reproducible increase in mutant*	See peer review proposed in 2(4) See also 2(16), 2(18), 2(19), 2(20), 2(21) and 2(31)

			frequency. *Biological relevance of the results should be considered first. Statistical methods may be used as an aid in evaluating the test results. Statistical significance should not be the only determining factor for a positive response*". Addressed.	
2(18)	3.1.3 *In vitro* mammalian gene mutation studies	Applicant (Aminotriazine TF): The co-RMS has referred to the assay performance criteria outlined in Clarke (2009) as a basis for their opinion that this study cannot be considered clearly negative. 1) We do not agree that the criteria for a valid study (cloning efficiency and negative and positive control responses) should be applied in the interpretation of the test substance results. The purpose of the performance criteria is to ensure that the assay is responding properly. 2) The co-RMS notes the response at 100 and 150 µg/ml was not replicated between the two cultures and the apparent increase in mutant frequency is driven by one replicate in each case. The mutant frequency of each of those cultures was ≤ 50 mutants/10^6 cells, the threshold noted above by Li et a. (1988).	RMS June 2018: See our response in 2(17) on the use of threshold values. Addressed. Co-RMS June 2018: The basis for our opinion that this study cannot be considered clearly negative is reported in 2(17). Addressed.	See peer review proposed in 2(4) See also 2(16), 2(17), 2(19), 2(20), 2(21) and 2(31)

		Further explanation: In their second paragraph, the co-RMS states "A concentration-dependent increase in mean mutant frequency was observed at the two highest doses tested … in the absence of metabolic activation. These values were clearly higher than the range of laboratory historical control data … The increased mutant frequency was only observed in one out of the two replicates. It is noted that, amongst the criteria for a valid test described in the study report, the following is reported: 'The positive control must induce a frequency of at least 3 times that of the solvent control and must exceed 40 mutants per 10^6 clonable cells.' These criteria set for positive controls were partially met at the dose levels of 100 and 150 µg/ml …"		
2(19)	Toxicology and metabolism: Genotoxicity	DE: DE considers IN-A4098 to have tested positive for mutagenicity in the absence of S-9 metabolic activation after 24hr exposure in the study by Clarke (2009). While the study was conducted according to OECD TG476 from 1997 and assessment according to the criteria at the time led to an equivocal result, reassessment according to the newer version	RMS June 2018: To conclude that an increase is concentration-related requires that a statistically significant result is obtained when the data is analysed with a trend test. This is explicitly stated in paragraph 39 (point b) of OECD 476 (v2016): "*the increase is concentration-related when evaluated with an appropriate trend test*". In OECD 476 (v1997) it is stated that a	See peer review proposed in 2(4) See also 2(16), 2817), 2(18), 2(20), 2(21) and 2(31)

		(2016) leads to a clearly positive result, namely: A) A dose-response relationship was observed; B) a statistically significant trend was found ($p<0.0001$, Cochrane-Armitage) and C) the mutation frequencies were outside the historical control data range (0-16.9, mean 3.7).	concentration-related increase is one criterion for determining a positive result. Nothing is mentioned about using a trend test, but it is the view of the RMS that statistical significance is required for establishing a trend. Otherwise any increase with increasing concentration observed in studies with low power could be argued to reflect a real positive trend. In this study statistical significances could not be established, neither for single test groups compared with the negative control group nor for the analyses of trend. DE states that a statistically significant trend was observed when the data was analysed with the Cochrane-Armitage test. However, it is important to include variation between replicate cultures in the statistical analysis of results from this type of study, as pointed out in Arlett et al. (1989) by stating: "*Any statistical analysis based only on the within-culture variation will therefore be likely to be spuriously sensitive and yield false significances.*" Statistical analyses considering the variability between replicate cultures have been performed both by the RMS and by [redacted] (2017). No statistical significances could be established in either of the analyses. Consequently, we do not agree with the conclusions made by DE. There was no concentration-related or reproducible increase in mutant frequency and, therefore, the

			result was negative both according to the criteria of OECD 476 (v1997) and OECD 476 (v2016). Addressed. <u>Co-RMS June 2018:</u> As the concentration-related increased mutant frequency was not reproducible in the second replicate, we rather considered the results of this study as equivocal. Addressed.	
2(20)	3.1.3 *In vitro* gene mutation assay	SI: Regarding the HPRT/CHO study by Clarke (2009) we agree with the opinion of the Co-RMS, that the study result can not be considered negative. We agree that the power of the study to detect chemicals inducing gene mutations is lower due to lower number of spontaneous mutations in the control group (compared to the spontaneous mutations frequency as proposed in the later version of the OECD guidance) and variability of study results. However, we do not support excluding this study from WoE analysis. Regarding the tk/L5178 study by Woods (2011) we agree with the opinion of the RMS, which concludes that the study result is negative. Taking into account the evaluation criteria of the 2016	<u>RMS June 2018:</u> Regarding the results of the study by Clarke (2009) the point estimates of mutant frequencies indeed increased with concentration, but when the data were subjected to statistical analysis no statistically significant increase in mutant frequency could be established. This is not surprising, since the low number of cells used in this study reduced the statistical power of the test. Accordingly, the negative result cannot be considered reliable and therefore no reliable conclusion about the mutagenicity of triazine amine can be made from the results of this study. This is our main argument for not accepting the study. Note that the number of cells used was not sufficient neither according to the requirements of the 2016 version of OECD 476 nor the requirements of the 1997 version of OECD 476. For details, please refer to the response to	See peer review proposed in 2(4) See also 2(16), 2(17), 2(18), 2(19), 2(21) and 2(31)

		version of OECD 490, which can be applied to the study performed, the increase in mutation frequency is below the control frequency plus GEF. Namely, the GEF was introduced into the OECD 490 due to several false positive results obtained with this study.	comment 2(16). We would like to take the opportunity to also comment on the hypothetical situation where the results of the study would have been used to evaluate the mutagenic effect of triazine amine. Clearly, the variation in mutant frequencies between replicate cultures in this study was large. It is important to include such variation in the statistical analysis of results from this type of study, as pointed out in Arlett et al. (1989) by stating: *"Any statistical analysis based only on the within-culture variation will therefore be likely to be spuriously sensitive and yield false significances."* Statistical analyses of the results of the study considering the variability of mutant frequencies between replicate cultures have been performed both by the RMS and by [redacted] (2017). No statistically significant increases in mutant frequency could be established in either of the analyses, meaning that the probability is high that the concentration-related increases seen for the point estimates of mutant frequencies occurred by chance only. Therefore, if the study would have been included in the evaluation it would have been concluded to be negative. Regarding the study by Woods (2011) we thank you for your support, but see also 2(27).

			Addressed. Co-RMS June 2018: Thank you for your support regarding the results of the Clarke study. Regarding the Woods study, we considered that the biological relevance of the concentration-related increased mutant frequency, clearly outside the range of the historical control data at the highest dose level, cannot be excluded, although slightly below the negative control + GEF. Addressed.	
2(21)	3.1.3 *in vitro* mammalian gene mutation study in CHO cells and mouse lymphoma test, pag. 10 and 12 respectively	EFSA: a concentration-related increase in gene mutations was observed in the experiment without metabolic activation. However, the study did not test a sufficient number of cells and therefore the RMS and also [redacted] 2017 concluded the study should not be included in the weight of evidence analysis of potential mutagenicity of triazine amine. The mouse lymphoma (L5178Y cells) test produced equivocal results (EFSA Conclusions of both thifensulfuron-methyl and metsulfuron-mehtyl) which were considered negative after re-assessment of the data by RMS and [redacted]2017. However	RMS June 2018: To conclude that an increase is concentration-related requires that a statistically significant result is obtained when the data is analysed with a trend test. This is explicitly stated in paragraph 39 (point b) of OECD 476 (v2016): "*the increase is concentration-related when evaluated with an appropriate trend test*". The comment gives the impression that EFSA finds it appropriate to consider that a concentration-related increase was observed just because the point estimates of mutant frequencies increased with increasing concentration and that no statistical analysis is necessary to establish this effect. The RMS certainly does not	See peer review proposed in 2(4) See also 2(16), 2(17), 2(18), 2(19), 2(20) and 2(31)

		EFSA agrees with Co-RMS that both the studies cannot be considered clearly negative and they were both equivocal in the absence of metabolic activation.	agree with this approach. Furthermore, since the statistical analyses considering the variability between replicate cultures (which is important, see the response to comment 2(19)) showed that there was no statistically significant concentration-related increase in mutant frequency, the RMS does not agree with the conclusion by EFSA on the gene mutation study in CHO cells (Clarke, 2009). Regarding the low cell number, see also 2(16). Finally, a minor remark; the low number of cells was observed by the RMS - not by [redacted](2017). With regard to the MLA study (Woods, 2011b), see 2(27). Addressed. <u>Co-RMS June 2018:</u> Thank you for your support. Addressed.	
2(22)	3.1.3 and 4.3.3 – *In vitro* MCGM studies – Woods, 2011b	UK: A re-evaluation of the Woods (2011b) MLA/TK study (originally considered equivocal) in accordance with the criteria of the revised guideline (OECD 490, 2016) shows that it is negative when tested up to the limit of solubility in DMSO, a concentration which was achieved by extremely aggressive measures and which is likely to represent a	<u>RMS June 2018:</u> Thank you for your support, but see also 2(27). Addressed. <u>Co-RMS June 2018:</u> According to OECD 476 (1997) in place at the time the study was conducted: "*There are several criteria for determining a positive result, such as a concentration-related, or a*	It is noted that according to RMS and UK, the *in vitro* mouse lymphoma study by Woods (2011b) was a reliable negative study, while according to EFSA and Co-RMS the results were not clearly negative. Peer review is proposed to discuss if results of the *in vitro* mouse lymphoma study by Woods (2011b) could be considered negative according to the

		suspension rather than a true solution. We agree with the RMS that this is a reliable negative study.	*reproducible increase in mutant frequency.* *Biological relevance of the results should be considered first. Statistical methods may be used as an aid in evaluating the test results. Statistical significance should not be the only determining factor for a positive response*". We considered that the biological relevance of the concentration-related increased mutant frequency, clearly outside the range of the historical control data at the highest dose level, cannot be excluded, although slightly below the negative control + GEF. The results of this study are therefore not clearly negative and should be considered equivocal at 24 hours without metabolic activation. Addressed.	criteria in OECD TG476 from 1997 or not clearly negative according to the latest (2016) version of the OECD TG476. See also 2(23), 2(24), 2(26), 2(27) and 2(28)
2(23)	3.1.3 *In vitro* mammalian gene mutation studies	Applicant (Aminotriazine TF): We agree with the conclusion by the RMS that the MLA study by Woods (2011) complies with a clearly negative result as defined by OECD 490 (2016) and which also agrees with the conclusion by Slovenia (2017).	RMS June 2018: Thank you for your support, but see also 2(27). Addressed. Co-RMS June 2018: Please see 2(22). Addressed.	See peer review proposed in 2(22) See also 2(24), 2(26), 2(27) and 2(28)
2(24)	3.1.3 *In vitro* mammalian gene mutation studies	Applicant (Aminotriazine TF): We disagree with the conclusion by the co-RMS that the MLA study by	RMS June 2018: We agree with reviewer that from a scientific viewpoint the development of	See peer review proposed in 2(22) See also 2(23), 2(26), 2(27) and 2(28)

		Woods (2011) is equivocal on the basis of OECD TG 476 (1997). Since 1997, significant review of the MLA was undertaken by international experts, and specific criteria for the performance and evaluation of the assay were adopted. The Woods (2011) study was interpreted, as noted in the study report, in accordance with the recommendations of Moore et al., 2006, which was the state of the art at the time of the study. These criteria were put in place to address the large number of false positive results that were occurring in the assay.	test guidelines should be taken into account when evaluating older studies, at least the most important revisions of the guidelines must be considered. Addressed. <u>Co-RMS June 2018:</u> Please see 2(22). Addressed.	
2(25)	3.1.3 *In vitro* mammalian gene mutation studies	Applicant (Aminotriazine TF): Regarding the MLA study by Woods (2011), we note that the apparent increase in mutant frequency at the highest concentration tested (308 µg/ml) is principally driven by a marked decrease in cell survival (expressed as Relative Total Growth or RTG) with only a minimal increase in actual number of mutants. Therefore, what appears to be an increase in mutant frequency between 154 µg/ml and 308 µg/mL, is attributed in part to the calculation of mutant frequency based on cell survival.	<u>RMS June 2018:</u> OECD 490 (v 2016) states that if the maximum concentration is based on cytotoxicity, the highest concentration should aim to achieve between 20 and 10% RTG (Relative Total Growth) for the MLA. In the present study excessive cytotoxicity was not observed, as shown by the RTG which was 71 and 54% at the concentration of 154 and 308 µg/mL, respectively. Addressed. <u>Co-RMS June 2018:</u> Agrees with the RMS. Addressed.	Addressed
2(26)	3.1.3 *In vitro*	Applicant (Aminotriazine TF): We do	<u>RMS June 2018:</u>	See peer review proposed in 2(22)

	mammalian gene mutation studies	not agree with the co-RMS that Woods (2011) should be considered equivocal because at the highest tested concentration the mean mutant frequency exceeded the range of laboratory historical controls. As the co-RMS notes, the mean mutant frequency at that concentration was below the negative control plus the GEF. Although we acknowledge that comparison to historical controls is valuable and used in the evaluation of results from other types of *in vitro* mammalian gene mutation assays, this is not the case for the MLA (OECD 490, 2016). Interpretation of the study findings based on whether mutant frequencies exceeded the negative control plus the GEF has been adopted to ensure that any increase in mutants is biologically relevant.	The RMS agrees with reviewer that the MLA study (Woods, 2011b) should be evaluated using the criteria of the revised OECD 490 (2016). See also 2(27). Addressed. Co-RMS June 2018: Please see 2(22). Addressed.	See also 2(23), 2(24), 2(27) and 2(28)
2(27)	3.1.3 , *in vitro* mouse lymphoma test, pag. 12	EFSA: in [redacted], 2017 the conclusion "equivocal" in regards to the results in the mouse lymphoma test in study by Woods (2011b) resulted from incorrectly applying the criteria for a negative response according to OECD TG 490, which are no increase in mutant frequency in respect to the negative control plus the Global Evaluation Factor, or no-	RMS June 2018: From the various comments it is clear that the criteria in §64 of OECD TG 490 are unclearly written and therefore results in different interpretations. Criteria for a clearly negative result (§64): "*Providing that all acceptability criteria are fulfilled, a test chemical is considered to be clearly negative if, in all experimental conditions examined*	See peer review proposed in 2(22) See also 2(23), 2(24), 2(26) and 2(28)

		concentration-related response observed. According to [redacted] 2017, it is only required that one of these two criteria are met to consider a result to be negative. A concentration-related increase in gene mutations was observed and EFSA agrees with Co-RMS that this is a biological criterion that should be considered more relevant than the increase in mutant frequency in respect to controls.	*(see paragraph 33) there is no concentration related response or, if there is an increase in MF, it does not exceed the GEF. The test chemical is then considered unable to induce mutations in this test system."* In our evaluation (April, 2018) the interpretation of the criteria was based on a presumed meaning of the word "or" which led to the conclusion that a result should be considered clearly negative if there is either no concentration-related response or an increase in mutant frequency (MF) which does not exceed the GEF. An alternative interpretation emerges if the text is read in a different way and the two concepts of the guideline [(i) increase in MF and (ii) concentrated-related increase/response in MF] are taken into account with the purpose to distinguish between effects seen as (i) an increase in any single treated group above the concurrent negative control exceeding the GEF, i.e. pairwise comparisons, and (ii) a concentration-related positive trend in any experiment established with a trend test. The alternative meaning of §64 would then be: *Providing that all acceptability criteria are fulfilled, a test chemical is considered to be clearly negative if, in all experimental conditions examined (see paragraph 33) there is no concentration related*

			response or, in the event there is an increase in MF in any single treated group above the concurrent negative control (pairwise comparisons), *it does not exceed the GEF. The test chemical is then considered unable to induce mutations in this test system.* This interpretation would mean that the criteria are composed of two independent parts. In Moore et al. (2006), referred to in OECD TG 490 (2016) regarding evaluation of results from the MLA, the following statement is given: A test agent response is clearly negative if *both* the trend analysis and the GEF are negative. Moore et al (2006) also states that an appropriate statistical trend test should be applied to determine whether there was a dose-related increase in MF. We believe that it is less fruitful to discuss further the correct interpretation of the criteria in §64 of OECD 490 in this context, since the issue is not unique for triazine amine. Regardless of the above discussion, it is possible to conclude that a result is positive or negative even if the criteria for a clearly positive or clearly negative result are not met. This is indicated in the Overview of the set of OECD Genetic Toxicology Test Guidelines and updates performed in 2014-2015 (OECD Series of Testing & Assessment No. 238, 13-Jul-2016), in which the

			following is stated: "*As outlined above, the revised/new TGs provide criteria for results that are clearly positive or negative. If the response is neither clearly negative nor clearly positive the TGs recommend that expert judgment be applied. Test results that do not meet all the criteria may also be judged to be positive or negative without further experimental data, but they need to be evaluated more closely before any final conclusion is reached.*" In the present case, Woods (2011b) did not report any trend test (as recommended in OECD TG 490) for the 24-h non-activated (-S9) system. To allow a more close evaluation of the results and to present a way forward, the RMS suggests that the applicant should be given the opportunity to submit a trend analysis of the results in Woods (2011b), in particular considering that the OECD 490 guideline was updated in 2016, which among other things involved revision of the criteria for evaluation of results. We suggest that the analysis should be based on the recommendations in Robinson et al: Statistical evaluation of bacterial/mammalian fluctuation test (In: Kirkland (Ed.) Statistical evaluation of mutagenicity test data, Cambridge Univ. Press, 1989). The RMS could not carry out such a trend test because it is recommended that a laboratory-specific heterogeneity factor

			(ratio of variances from several experiments to the theoretical binomial variances) is used (section 4.2.2 in Robinson et al). Co-RMS June 2018: Thank you for your support. Addressed	
2(28)	Toxicology and metabolism: Genotoxicity	DE: Similarly at the time the study by Woods (2011b) was conducted the results could be considered negative according to the criteria in OECD TG476 from 1997. However, according to the latest (2016) version of the OECD TG476 the following criteria must be fulfilled for a result to be considered clearly negative: a) none of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control; b) there is no concentration-related increase when evaluated with an appropriate trend test; c) all results are inside the distribution of the historical negative control data (e.g. Poisson-based 95% control limit; see paragraph 33), Given that there is a clear if modest dose response relationship for the 24 hr treatment in the absence of S-9, the mutation frequency at the highest dose is outside the	RMS June 2018: We refer to the Overview of the set of OECD Genetic Toxicology Test Guidelines and updates performed in 2014-2015 (OECD Series of Testing & Assessment No. 238, 13-Jul-2016): "*Since the last round of TG revisions in 1997, new TGs have been adopted: […]and finally, TG 490 (*in vitro *mammalian cell gene mutation assays using the thymidine kinase (TK) gene [Mouse Lymphoma Assay (MLA) and TK6 test] approved in 2015. Because of the acceptance of a new TG (TG 490) that includes both the MLA and TK6 tests, TG 476 was revised and updated, and now includes only the* in vitro *mammalian cell gene mutation tests using the hypoxanthine guanine phosphoribosyl transferase (Hprt) locus and xanthine-guanine phosphoribosyl transferase transgene (xprt) gene.*" The criteria cited by the reviewer (from §40 in OECD TG No 476, 2016) are therefore not relevant for the interpretation of Woods (2011b). The relevant criteria to identify clearly	See peer review proposed in 2(22) See also 2(23), 2(24), 2(26) and 2(27)

		historical control range and no statistical analysis was performed, DE considers this study not to be clearly negative, but equivocal.	negative results in the mouse lymphoma assay are those in §64 of OECD TG 490 (2016). Addressed Co-RMS June 2018: Thank you for your support. Addressed	
2(29)	3.1.3, *in vitro* mammalian gene mutation study in mouse lymphoma by Lloyd 2016, pag. 13	EFSA: as RMS pointed out, the highest concentration tested in this study was significantly lower than the comparatively similar top concentration tested in the other *in vitro* studies in mammalian cells. Therefore the results of the study are considered not relevant.	RMS June 2018: Thank you for your support. Addressed Co-RMS June 2018: Thank you for your support. Addressed	Addressed
2(30)	3.1.3 and 4.3.3 – *In vitro* MCGM studies – Lloyd, 2016a	UK: This was a new MLA/TK study also submitted to the UK for the purposes of the confirmatory information requirements of thifensulfuron-methyl. An evaluation of this study in accordance with the criteria of the revised guideline (OECD 490, 2016) shows that it is negative when tested up to the alleged true limit of solubility of triazine amine in DMSO. We note that the analysis performed by the RMS suggests that the highest concentration tested in this study may have not been maximised. However, we disagree that there is sufficient evidence to consider the study completely unreliable. As a minimum it should be	RMS June 2018: The low concentrations tested is a result of the "maximum practicable concentration" obtained in DMSO, which is much lower in this study than what was reported in other available studies. We maintain our view that, due to the low concentrations, the negative result should not be considered reliable. Addressed Co-RMS June 2018: Agrees with the RMS. Addressed	Addressed

		considered supportive/supplementary of the other negative studies.		
2(31)	Conclusion on gene mutation in mammalian cells	AT: AT agrees with the Co-RMS France that study Clarke (2009) and Woods (2011) showed equivocal and not negative results. Since in the study Lloyd (2016) the concentrations tested were too low, the concerns for gene mutation of triazine amine in mammalian cells cannot be ruled out with confidence.	RMS June 2018: Regarding Clarke (2009), see in particular 2(16) and 2(21), and also 2(17), 2(18), 2(19), 2(20). Regarding Woods (2011b), see in particular 2(27), and also 2(20), 2(21), 2(22), 2(23), 2(24), 2(25), 2(26) and 2(28). Addressed Co-RMS June 2018: Thank you for your support. See also 2(55). Addressed	See peer review proposed in 2(4) See also 2(16), 2(17), 2(18), 2(19), 2(20) and 2(21)
2(32)	3.1.4, *in vitro* DNA damage and repair	EFSA: a summary of material and methods and main results of such studies should be further detailed.	RMS June 2018: We do not agree. The task was to evaluate the weight of evidence assessment submitted as confirmatory data ([redacted] 2017). For that purpose it was necessary to check each study carefully in order to judge whether each study was acceptable and whether or not each study should be included in the weight of evidence assessment. This was done and the two *in vitro* studies on DNA damage and repair (Hertner 1988; Meyer 1988) were considered acceptable and as such possible to include in a WoE analysis. However, the weight of these two indicator studies is negligible in	Noted

			the WoE analysis, since negative results from *in vitro* studies on permanent changes of the DNA, i.e. mutations, are available. This was stated in section 4.3.4. It is therefore not considered relevant to present material and methods and results for these two studies. Addressed Co-RMS June 2018: Agrees with the RMS. Addressed	
2(33)	3.1.4 and 4.3.4 – *in vitro* UDS studies	UK: These negative UDS studies are acceptable and, although indicator tests only, contribute to the overall genotoxicity WoE of triazine amine and further support the negative results obtained in the other *in vitro* tests in mammalian cells. Therefore, we disagree with the RMS and Co-RMS that these studies should be excluded from the WoE analysis. As a minimum, they should be considered supportive/supplementary of the other negative *in vitro* tests in mammalian cells.	RMS June 2018: Results of mutagenicity tests are generally of higher significance than indicator tests. If the available UDS studies would have been positive the results would not have influenced the conclusion that triazine amine is not mutagenic. Nor do they significantly contribute to the conclusion when, as in the present case, the results are in agreement with the results of mutagenicity tests. We would therefore not designate the negative USD studies as supportive but could agree to a wording saying that the results do not contradict the results of the mutagenicity studies. See also 2(47). Open point for the RMS to indicate in a revised report (section 4.3.4) that the results of the USD studies do not contradict the results of the	Addressed See also 2(47)

			mutagenicity studies. Co-RMS June 2018: Agrees with the RMS. Addressed	
2(34)	3.1.4 *In vitro* DNA damage and repair	Applicant (Aminotriazine TF): We agree with the RMS and co-RMS that the two *in vitro* UDS studies conducted in primary rat hepatocytes (Hertner, 1988) and in human fibroblasts (Meyer, 1988) are negative.	RMS June 2018: Thank you for your support. Addressed Co-RMS June 2018: Thank you for your support. Addressed	Addressed.
2(35)	3.1.5, *in vivo* chromosome aberration study	EFSA: a summary of material and methods and main results of such studies should be further detailed. As pointed out by RMS and Co-RMS, no evidence of bone marrow exposure was provided, although recognising the tested dose was the highest applicable dose. It is therefore questioned if clastogenicity *in vivo* is considered adequately assessed by this study.	RMS June 2018: We do not agree to present in detail material and methods and results for the *in vivo* chromosome aberration study. The task was to evaluate the weight of evidence assessment submitted as confirmatory data ([redacted]). For that purpose it was necessary to check each study carefully in order to judge whether each study was acceptable and whether or not each study should be included in the weight of evidence assessment. This was done and this *in vivo* study was considered as less useful for the WoE assessment since exposure of the bone marrow was not demonstrated. This was stated in section 4.3.5. It is therefore not considered relevant to present material	Noted.

			and methods and results for this study in this context. We agree that clastogenicity *in vivo* has not been adequately assessed by this study - but since triazine amine did not induce chromosome aberrations in mammalian cells *in vitro* a follow-up study of this endpoint *in vivo* is not required. Addressed Co-RMS June 2018: Agrees with the RMS. Addressed	
2(36)	3.1.5 and 4.3.5 – *In vivo* chromosome aberration study	UK: We disagree with the RMS and co-RMS that the available negative *in vivo* clastogenicity study in hamsters ([redacted] 1988) is unreliable because of lack of proof of bone marrow exposure. This study tested the high dose of 3200 mg/kg bw (higher than the recommended limit dose) based on the outcome of a tolerability test which employed a dose of 5000 mg/kg bw. The dose of 3200 mg/kg bw was selected as it was the highest dose in the tolerability test which caused no death. In addition, based on the results of an LD50 study in rats, at 2000 mg/kg bw males showed severe clinical signs of toxicity and females showed mortality at 1000	RMS June 2018: We agree that a very high dose was tested in this study. However, a conclusion that the bone-marrow was sufficiently exposed should not be based on assumptions but should be demonstrated by the information specified in OECD 475. This information is not available and therefore our view is that the negative result of the study is unreliable. Since triazine amine did not induce chromosome aberrations in mammalian cells *in vitro* we certainly agree that a follow-up study of this endpoint *in vivo* is not required. Addressed Co-RMS June 2018:	Addressed.

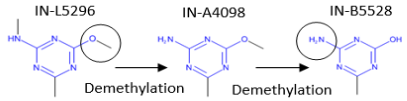
		mg/kg bw. Therefore, it is most likely that the massive dose of 3200 mg/kg bw in hamsters caused systemic toxicity (even if not explicitly reported in the study), indicating systemic exposure and hence bone marrow exposure. Therefore, we are of the opinion that this study is a reliable negative study. It should also be noted that triazine amine is not clastogenic *in vitro*; hence *in vivo* follow-up is not required.	Agrees with the RMS. Addressed	
2(37)	3.1.5 *In vivo* chromosome aberration study	Applicant (Aminotriazine TF): The RMS and co-RMS note that data confirming bone marrow exposure was not presented in support of [redacted] (1988). As noted in [redacted] (2017), this data is not necessary to conclude that triazine amine is not clastogenic as the sum of the reliable *in vitro* chromosome aberration studies confirms the absence of clastogenic potential. We suggest that a statement to this effect be included in the confirmatory data review.	RMS June 2018: A statement could be added to clarify that data from an *in vivo* study on chromosome aberrations is not required to conclude that triazine amine does not induce chromosome aberrations, since all acceptable *in vitro* studies on this endpoint were negative. Open point for the RMS to indicate in a revised report that data from an *in vivo* study on chromosome aberrations is not required to conclude that triazine amine does not induce chromosome aberrations. Co-RMS June 2018: Agrees with the RMS. Addressed	Addressed.
2(38)	Toxicology and metabolism:	DE: DE also agrees that all *in vitro* chromosomal aberration studies	RMS June 2018: The first comment is confusing; we	Addressed.

	Genotoxicity	by [redacted](1987) were valid and none demonstrated a clastogenic potential of IN-A4098 in bacteria. The results of the *in vivo* chromosomal aberration study by Strasser (1988) are, however, inconclusive as exposure of the bone marrow to IN-A4098 was not/cannot be demonstrated. Nonetheless, DE considers the clastogenic potential of IN-A4098 in mammalian cells to have been sufficiently investigated.	assume that the reviewer meant to say that they agree that all *in vitro mammalian* chromosome aberration studies were valid (except the *single* study carried out by Dollenmeier, 1987), and negative. With regard to the *in vivo* study ([redacted], 1988) we agree with the reviewer. Addressed <u>Co-RMS June 2018:</u> Agrees. Addressed	
2(39)	3.1.6 Quantitative structure activity relationship (QSAR), pag. 16	EFSA: EFSA agrees with RMS and disagrees with [redacted]. 2017 that QSAR analyses are relevant for the evaluation of the potential mutagenicity of triazine amine.	<u>RMS June 2018:</u> Thank you for your support. Addressed <u>Co-RMS June 2018:</u> Thank you for your support. Addressed	Addressed.
2(40)	3.1.6 – QSAR analysis	UK: Although we agree that the QSAR analysis has less value/weight when there is experimental data, the negative results shown by the QSAR evaluation still contribute to the WoE assessment. As a minimum, it should be considered supportive/supplementary of all the other negative tests (*in vitro* and *in vivo*) available on triazine amine.	<u>RMS June 2018:</u> Since information from QSAR analyses are not considered when experimental data are available we would not designate such analyses as supportive. However, we could agree to a wording saying that the results of the QSAR analyses do not contradict the results of the mutagenicity studies. Open point for the RMS to indicate in a revised report (section 3.1.6) that the	Addressed See also 2(41)

			results of the QSAR analyses do not contradict the results of the mutagenicity studies. Co-RMS June 2018: Agrees with the RMS. Addressed.	
2(41)	Point 3.1.6 Quantitative structure activity relationship (QSAR)	Applicant (Aminotriazine TF): We agree in most cases when test data are available, those data override (Q)SAR predictions. However, we do not agree that (Q)SAR predictions are useless in this specific case and should be ignored. (Q)SAR predictions are intended to predict toxicity including genotoxicity in the absence of data. However, that does not mean that (Q)SAR predictions should automatically be ignored for data-rich compounds. Rather, when the interpretation of existing data is questioned, such predictions can be used to provide further weight of evidence. In fact, this is the recommendation of EFSA in its recent clarification on genotoxicity (November 2017) in which all lines of evidence should be considered including (Q)SAR and read-across from structurally similar molecules particularly in cases where there may be some residual uncertainties (excerpt provided in next column [RMS: For clarity we	RMS June 2018: During the interpretation and evaluation of the available data, the RMS concluded that a weight of evidence analysis of the potential mutagenicity of triazine amine could be based on the available *in vitro* studies. Therefore, it was not necessary to consider QSAR predictions to arrive at a conclusion. Indeed, the QSAR predictions did not contradict the results of the mutagenicity studies, but they are not needed as supporting information in the weight of evidence analysis performed by the RMS and were therefore omitted from the analysis. See Open point in 2(40). Co-RMS June 2018: (Q)SAR analysis should not be used to dismiss a positive/equivocal results of an experimental test. Addressed.	See 2(40)

		inserted this text below]). Further explanation: Excerpt from EFSA Clarification of Some Aspects Related to Genotoxicity Assessment (November 2017) regarding WoE and use of additional information to reduce uncertainty: "In case it is not possible to conclude on genotoxicity with confidence, the assessor may in a second step, take into consideration all available data that may assist in reducing the uncertainty, including studies on mode of action, read-across from structurally related substances and predictions from QSAR models within their applicability domain. Information on carcinogenicity testing and reproductive toxicity testing, and other information such as ADME may also assist in reducing the uncertainty."		
2(42)	3.1.6 Quantitative structure activity relationship (QSAR)	Applicant (Aminotriazine TF): The RMS notes that read-across of data from structurally similar compounds is not relevant since data on triazine amine is available. We disagree in this specific case as there is some uncertainty by the co-RMS regarding the Clarke (2009) and Woods (2011) studies. IN-B5528 is down-stream metabolite of triazine amine	<u>RMS June 2018:</u> During the interpretation and evaluation of the available data, the RMS concluded that a weight of evidence analysis of the potential mutagenicity of triazine amine could be based on the available *in vitro* studies. Therefore, it was not necessary to consider read-across of data to arrive at a conclusion. The	Addressed.

		formed through the process of demethylation and is a metabolite of thifensulfuron methyl. This metabolite produced negative results in the MLA (maximum concentration 240 µg/ml). Further, a recently completed MLA with IN-L5296, an upstream metabolite of triazine amine and metabolite of tribenuron methyl, also produced negative results (maximum concentration 1670 µg/ml). The combination of MLA studies with IN-B5528 and IN-L5296 cover the functional groups contained in triazine amine and therefore are particularly relevant for concluding the *in vitro* mammalian gene mutation potential of triazine amine (structures shown in adjacent column [RMS: For clarity we inserted text and structures below]). Further, the maximum concentrations tested were near or greater than those tested with triazine amine. Further explanation: Excerpt from EFSA Clarification of Some Aspects Related to Genotoxicity Assessment (November 2017) regarding WoE and use of additional information to reduce uncertainty: "In case it is not possible to conclude on genotoxicity with	RMS also note that Member States and EFSA have not as yet agreed on a conclusion based on the MLA studies on IN-B5528 and IN-L5296. Addressed. <u>Co-RMS June 2018:</u> Agrees with the RMS. Addressed.	

		confidence, the assessor may in a second step, stake into consideration all available data that may assist in reducing the uncertainty, including studies on mode of action, read-across from structurally related substances and predictions from QSAR models within their applicability domain. In formation on carcinogenicity testing and reproductive toxicity testing, and other information such as ADME may also assist in reducing the uncertainty." As noted in [redacted] (2017), the electronic/topological similarity score comparing triazine amine (IN A4098) to IN-B5528 is 89%. Using the same approach, the structural similarity score between IN A4098 and IN-L5296 is 90%. The results indicate close structural matches and that the negative test results from IN-B5528 and IN L5296 can be extrapolated to IN-A4098.  The circles identify the functional groups contained in IN-A4098 covered by IN-L5296 and IN-B5528.		
2(43)	3.1.7 Metabolism, pag. 17	EFSA: EFSA considers that no conclusion on the genotoxicity of	RMS June 2018: Thank you for your support.	Addressed.

		triazine amine could be made from *in vivo* genotoxicity studies and from rodent carcinogenicity studies of parent molecules. Therefore EFSA agrees with RMS and disagrees with [redacted], 2017	Addressed. Co-RMS June 2018: Thank you for your support. Addressed.	
2(44)	3.1.7 Metabolism	Applicant (Aminotriazine TF): We maintain our position that the outcome of carcinogenicity studies is relevant for assessing *in vivo* genotoxicity. As explained in [redacted] (2017), rodent carcinogenicity studies have served as the basis for assessing the predictivity of *in vitro* and *in vivo* genotoxicity assays for decades. In fact, the basis of EFSA 2011 opinion on genotoxicity and its recommended test battery is predicated on the outcome of rodent bioassays. We have not suggested that carcinogenicity tests should replace *in vivo* genotoxicity assessments, but that the outcome of the studies with the parent SUs from which triazine amine is a metabolite in rodents, should be included in an overall WoE evaluation, as noted in the recent EFSA clarification on genotoxicity (November 2017). Further explanation: Excerpt from EFSA Clarification of	RMS June 2018: The reason why rodent carcinogens in the early days were chosen as substances for assessing predictivity and specificity of genotoxicity tests and test batteries was the increasing knowledge that many carcinogens have mutagenic properties. Consequently, the probability to have mutagens in a group of carcinogens was higher than to have mutagens in a group of randomly selected substances. The issue was to determine how efficient the genotoxicity tests were to detect the mutagens among the carcinogens. This has nothing to do with a reverse mode of detection, i.e. that carcinogenicity tests results could predict mutagenicity. Carcinogenicity tests are not sensitive enough to reliably distinguish a mutagen from a non-mutagen. Addressed. Co-RMS June 2018: Agrees with the RMS. Addressed.	Addressed.

		Some Aspects Related to Genotoxicity Assessment (November 2017) regarding WoE and use of additional information to reduce uncertainty: "In case it is not possible to conclude on genotoxicity with confidence, the assessor may, in a second step, take into consideration all available data that may assist in reducing the uncertainty, including studies on mode of action, read-across from structurally related substances and predictions from QSAR models within their applicability domain. Information on carcinogenicity testing and reproductive toxicity testing, and other information such as ADME may also assist in reducing the uncertainty."		
2(45)	3.1.7 Metabolism	Applicant (Aminotriazine TF): We do not agree with the statement that metabolites "which could be considered as studied in the toxicity studies performed on an active substance are those detected at 10% or more of the administered dose in the urine in ADME studies." This statement is based on draft 2016 EFSA guidance on residue definition, and that document has not been yet noted.	<u>RMS June 2018:</u> The reviewer refers to a statement of the Co-RMS. Regardless of the recommendations of the guidance mentioned by the reviewer, the RMS maintains the position that the levels of triazine amine in urine were not sufficiently high to comply with the OECD requirements regarding dose levels of *in vivo* genotoxicity studies. Addressed. <u>Co-RMS June 2018:</u> We agree that the draft EFSA guidance	Addressed.

			on residue definition has not yet been noted. Nevertheless, this statement represents the current practice and is not specifically related to this draft guidance. Addressed.	
2(46)	3.1.8, Overall conclusions presented in [redacted] 2017, pag. 18	EFSA: EFSA agrees with Co-RMS in considering the results *in vitro* mammalian cell mutation tests from Clarke 2009 (in CHO cells) and Woods 2011 (mouse lymphoma in L5178Y cells) as equivocal without metabolic activation. The study from Flugge 2011 (foetal hamster V79) was negative, while Lloyd 2016 (mouse lymphoma in L5178Y cells) was not considered reliable. Therefore, it can be concluded that no firm conclusion can be drawn concerning the gene mutation induction potential of triazine amine.	RMS June 2018: Regarding Flügge (2011b) and Lloyd (2016) we agree with the reviewer. Regarding Clarke (2009), see in particular 2(16) and 2(21), and also 2(17), 2(18), 2(19) and 2(20). Regarding Woods (2011b) see in particular 2(27), and also 2(20), 2(21), 2(22), 2(23), 2(24), 2(25), 2(26) and 2(28), Addressed. Co-RMS June 2018: Thank you for your support. Addressed.	Addressed.
2(47)	Toxicology and metabolism: Genotoxicity	DE: On page 18, under the heading "RMS Conclusion", it is worth mentioning the results of the two UDS mutation assays for the sake of completeness, but also that they are of little consequence for the Weight of Evidence (WoE) because according to the new EFSA guidance on the assessment of genotoxicity studies (EFSA Journal 2017;15(12):5113) a) negative results can only be	RMS June 2018: Not agreed, since the "RMS conclusions" on p. 18 were meant to respond only to the above stated overall conclusions on the studies, as presented in [redacted] (2017). The consequence for WoE assessment was not discussed for any of the other studies in this section so it would only be confusing to discuss it only for two of them. The consequence for the WoE assessment is discussed in proper	See 2(33)

		considered with caution and b) the UDS assay is of little relevance if the liver is not the target organ. This is mentioned later in the report under 4.3.4 Analysis of in vitro DNA damage and repair data, however it should also be mentioned here.	context in section 4.3.4. The See also 2(33). Addressed. Co-RMS June 2018: This comment refers to "RMS conclusions". Addressed.	
2(48)	4.2.1 General conditions and compilation of relevant data on exposure	Applicant (Aminotriazine TF): The RMS makes the statement that solubilization of triazine amine in DMSO would be expected to be virtually identical for all studies, provided that no special measures were made to maximise solubility. We do not agree with this statement as in the studies of Woods (2011) particularly aggressive measures were undertaken to solubilise triazine amine with excessive heating, sonication and vortexing.	RMS June 2018: That is exactly what we mean. The solubility in the Woods studies is high and special measures were made to maximise solubility. Addressed. Co-RMS June 2018: Agrees with the RMS. Addressed.	Addressed.
2(49)	4.2.4 Evaluation of data from the studies in the different categories, pag. 24	EFSA: EFSA agrees with the approach proposed by RMS to categorise the studies on the basis of exposure information available and to provide a weight of evidence approach to assess the genotoxicity of triazine amine.	RMS June 2018: Thank you for your support. Addressed. Co-RMS June 2018: Agrees. Addressed.	Addressed.
2(50)	Toxicology and metabolism: Genotoxicity	DE: On page 24, under 4.2.4 Evaluation of data from the studies in the different categories, it was stated that "Category I	RMS June 2018: EFSA Scientific Opinion (Scientific Committee, 2017) identifies different aspects to consider when evaluating	Peer review is proposed to agree upon the criteria to be used to evaluate the studies that can be included in the weight of evidence analysis.

		studies are non-informative for determining solubility limits in DMSO and culture medium, since the concentrations used in DMSO and culture medium were soluble. Therefore, the RMS considers that these studies should not be included in the evaluation of the potential mutagenicity of triazine amine. However, note that in the case the studies had produced reliable positive results for genotoxicity, they would have been accepted without having determined the solubility limits." The exclusion of studies when the results are negative, but not when they are positive is not very scientific. They should be included taking into account other relevant information such as solubility data reported elsewhere, regardless of the result. Moreover, DE agrees with the co-RMS conclusion on page 25 that the study by Meyer (1991) should still be included in the Weight of Evidence (WoE) assessment despite a lack of information concerning the solubility limits in the study.	negative test results, as compared to evaluation of positive test results. Test concentrations is one of the aspects which ECHA (2017) suggests should be taking into account when evaluating negative results (but not positive results). We believe there is good scientific reasons to do so, to make sure that the negative result was not due to use of too low test concentrations. By contrast, for a positive result it does not matter if the study was carried out far below the solubility limit of the test substance. With regard to Meyer (1991), it is stated that the highest concentration tested was the highest concentration soluble in culture medium. However, there is no information about at which concentration triazine amine was insoluble. Therefore, this is a Category 1 study that should not be included in the evaluation of the potential mutagenicity of triazine amine. Addressed. <u>Co-RMS June 2018:</u> Thank you for your support regarding the inclusion of the study by Meyer (1991) in the WoE. Addressed.	See also 2(52)
2(51)	4.2.5 RMS's overall conclusion regarding	SI: Thank you for overview of triazine amine solubility and doses used in	<u>RMS June 2018:</u> Thank you very much for your support.	Addressed.

	test concentrations	genotoxicity studies. We agree that sufficiently high doses were used in genotoxicity studies with exception of study by Lloyd (2016).	Addressed. Co-RMS June 2018: Thank you for your support. Addressed.	
2(52)	4.2.5 RMS's overall conclusion regarding test concentrations	Applicant (Aminotriazine TF): We agree with the co-RMS that the study by Meyer (1991) can be used in a WoE evaluation as not only were the highest concentrations tested within the range of other studies, but the study report indicates that triazine amine was tested to the maximum solubility limit in culture medium. Further explanation: Excerpt from Meyer (1991): "The highest concentration of CGA 150 829 tech. in DMSO (stock solution), soluble in culture medium, was 10 mg/ml. ….The respective solutions were added (1:100 to the cell cultures. The final concentration of the vehicle DMSO in the culture medium was 1%." Therefore, it can be concluded that 100 µg/ml was the highest soluble concentration in culture medium that could be achieved under the conditions of the study.	RMS June 2018: It is stated (in Meyer, 1991) that the highest concentration tested was the highest concentration soluble in culture medium. However, there is no information about at which concentration triazine amine was insoluble. Therefore, this is a Category 1 study that should not be included in the evaluation of the potential mutagenicity of triazine amine. Addressed. Co-RMS June 2018: Thank you for your support. Addressed.	See peer review proposed in 2(50)
2(53)	4.3.3 and 4.3.6 Analysis	Applicant (Aminotriazine TF): We do	RMS June 2018:	Addressed.

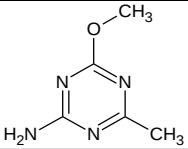
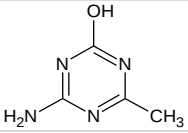
	of gene mutation data and General conclusion	not agree with the conclusions of the co-RMS regarding either Clarke (2009) or Woods (2011b), and thus we do not agree with their overall conclusion regarding *in vitro* gene mutation potential of triazine amine.	Noted. Addressed. Co-RMS June 2018: Please see 2(17) and 2(22). Addressed.	
2(54)	4.3.3 Analysis of *in vitro* gene mutation assay	SI: Our opinion is that the result of gene mutation study HPRT/CHO is equivocal, not negative. Thereafter the gene mutation potential of triazine amine can not be excluded.	RMS June 2018: Please see the response in particular to 2(16) and 2(21), and also 2(17), 2(18), 2(19) and 2(20). Addressed. Co-RMS June 2018: Thank you for your support. Addressed.	Addressed. See also 2(16), 2(17), 2(18), 2(19), 2(20) and 2(21)
2(55)	Toxicology and metabolism: Genotoxicity	DE: Therefore DE disagrees with the RMS that a) the in vitro mammalian cell mutagenicity study by Clarke (2009) was equivocal and b) the in vitro mammalian cell mutagenicity study by Woods (2011b) was negative. Rather, we agree with the co-RMS that the studies were positive and equivocal respectively. As a consequence, there are three valid studies assessing mammalian cell mutagenicity, one negative (Flügge, 2011b), one equivocal (Woods, 2011b) and one positive (Clarke, 2009). DE does not	RMS June 2018: In the reviewer's commenting table, this comment was placed after the comments now numbered as 2(19) and 2(28). We inserted this comment further down in the Reporting table since it relates to the overall conclusions. Since the conclusion of the reviewer was based on comments 2(19) and 2(28) we refer to our responses to those comments. Regarding Clarke (2009), see in particular 2(16) and 2(21), and also 2(17), 2(18), 2(19) and 2(20). Regarding Woods (2011b), see in particular 2(27), and also 2(20), 2(21),	See also 2(16)

		consider the mutagenic potential of IN-A4098 in mammalian cells to have been sufficiently investigated. A transgenic rodent assay according to the OECD TG488 would be the most effective means by which any genotoxic potential could be conclusively excluded.	2(22), 2(23), 2(24), 2(25), 2(26) and 2(28). Addressed. Co-RMS June 2018: Thank you for your support. The same conclusion was drawn by the co-RMS considering the study by Clarke (2009) as equivocal rather than positive. The need for an *in vivo* assay to conclude on the gene mutation potential of triazine amine is proposed to be discussed at an expert meeting.	
2(56)	4.3.6, general conclusion, pag. 28	EFSA: no firm conclusion can be drawn regarding the gene mutation potential of triazine amine from the evidence provided by the *in vitro* studies in mammalian cells and also on the basis of exposure information available and by the weight of evidence analysis provided. It is therefore considered that genotoxicity should be assessed in *in vivo* studies.	RMS June 2018: We do not agree with reviewer, see in particular our responses to 2(16), 2(21) and 2(27). The RMS does not agree that the issue of induction of gene mutations needs to be further addressed by performing an *in vivo* study. It is not ethically acceptable to propose an animal study. Instead, the RMS suggests an appropriate trend test on the results in Woods (2011b), see 2(27). Co-RMS June 2018: Thank you for your support. The need for an *in vivo* assay to conclude on the gene mutation potential of triazine amine is proposed to be discussed at an expert meeting. See 2(55)	See also 2(16)

2(57)	4.3.6 – Overall conclusion	UK: Overall, we agree with the RMS that triazine amine is not genotoxic. In our view, there is an abundance of data and different strands of evidence, all pointing out in the same direction. Although some individual studies have limitations and are not perfect, the weight of evidence overwhelmingly indicates that triazine amine is not genotoxic and that no further testing is required.	RMS June 2018: Thank you for support, but see also, in particular, 2(27). Co-RMS June 2018: We do not agree and consider that, by weight of evidence, no firm conclusion can be drawn regarding the gene mutation potential of triazine amine. See 2(55)	See 2(27) and 2(55)
2(58)	4.3.6 General conclusion	Applicant (Aminotriazine TF): After a detailed and thorough weight of evidence analysis, the RMS has concluded that there are no results supporting that triazine amine induces gene mutations or chromosome aberrations in mammalian cells *in vitro*. As a result, the genotoxicity of triazine amine can be concluded and no further information, particularly animal studies, is needed. The Aminotriazine Taskforce agrees with this conclusion.	RMS June 2018: Thank you for your support, but see also 2(27). Co-RMS June 2018: We do not agree and consider that, by weight of evidence, no firm conclusion can be drawn regarding the gene mutation potential of triazine amine. See 2(55)	See 2(27) and 2(55).
2(59)	4.3.6 General conclusion	SI: We agree that triazine amine did not induce gene mutations in bacteria or chromosomal aberrations in mammalian cells. However, our opinion is that the gene mutation potential of triazine amine can not be excluded and due to formation of this common metabolite from	RMS June 2018: Thank you for your support that triazine amine did not induce gene mutations in bacteria or chromosome aberrations in mammalian cells. Regarding your view regarding the potential of triazine amine to induce gene mutations in mammalian cells, please see the response in particular	See 2(27) and 2(55).

		several sulfonyl urea herbicides this issue should be further addressed.	to 2(16), 2(21) and 2(27), and also 2(17), 2(18), 2(19) and 2(20). See 2(27) <u>Co-RMS June 2018:</u> Thank you for your support. See 2(55)	

Appendix B – Used compound codes

Code/trivial name	Chemical name/SMILES notation	Structural formula
Triazine amine IN-A4098	4-methoxy-6-methyl-1,3,5-triazin-2-amine Cc1nc(N)nc(OC)n1 NXFQWRWXEYTOTK-UHFFFAOYSA-N	
IN-B5528	4-amino-6-methyl-1,3,5-triazin-2-ol Nc1nc(C)nc(O)n1 UUTHDVPZNWJUFV-UHFFFAOYSA-N	
IN-L5296	4-methoxy-*N*,6-dimethyl-1,3,5-triazin-2-amine Cc1nc(NC)nc(OC)n1 MNDSUSQBIDHEJU-UHFFFAOYSA-N