TECHNICAL REPORT

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

Keywords: acibenzolar-S-methyl, peer review, confirmatory data, risk assessment, pesticide, plant activator

Requestor: European Commission

Question number: EFSA-Q-2020-00261

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Summary

The approval of acibenzolar-S-methyl was renewed under Regulation (EC) 1107/2009 on 1 April 2016 by Commission Implementing Regulation (EU) 2016/389. It was a specific provision of the approval that the applicant was required to submit to the European Commission further information by 1 June 2017 as regards the relevance and reproducibility of the morphometric changes observed in the cerebellum of foetuses linked to exposure to acibenzolar-S-methyl and whether these changes may be produced via an endocrine mode of action. The information to be submitted shall include a systematic review of the available evidence assessed on the basis of available guidance (e.g. EFSA GD on Systematic Review methodology, 2010).

In accordance with the specific provision, the applicant, Syngenta, submitted an updated dossier in May 2017 as well as additional information in February 2019 in line with the EFSA/ECHA guidance for the identification of endocrine disruptors (2018), which was evaluated by the designated rapporteur Member State (RMS), France, 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 12 November 2019. The RMS collated all comments in the format of a reporting table, which was submitted to EFSA on 11 March 2020. 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, France, and presents EFSA's scientific views and conclusions on the individual comments received.

The confirmatory data are considered not addressed. During the commenting period the link between developmental neurotoxicity effects (DNT) and T-mediated endocrine effect, supported by the RMS, was still considered to be further discussed and an experts' consultation should be organised.

It is also proposed to discuss the assessment of the endocrine disrupting (ED) properties of acibenzolar-S-methyl both for humans and non-target organisms and which additional test are needed to conclude on the ED properties.

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1. Introduction

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

The approval of acibenzolar-S-methyl was renewed under Regulation (EC) 1107/2009[1] on 1 April 2016 by Commission Implementing Regulation (EU) 2016/389[2]. EFSA previously finalised a Conclusion on this active substance on 12 August 2014 in the EFSA Journal (EFSA, 2014).

It was a specific provision of the approval that the applicant was required to submit to the European Commission further information by 1 June 2017 as regards the relevance and reproducibility of the morphometric changes observed in the cerebellum of foetuses linked to exposure to acibenzolar-S-methyl and whether these changes may be produced via an endocrine mode of action. The information to be submitted shall include a systematic review of the available evidence assessed on the basis of available guidance (e.g. EFSA GD on Systematic Review methodology, 2010).

In accordance with the specific provision, the applicant, Syngenta, submitted an updated dossier in May 2017 as well as additional information in February 2019 in line with the EFSA/ECHA guidance for the identification of endocrine disruptors (2018), which was evaluated by the designated rapporteur Member State (RMS), France, in the form of an addendum to the draft assessment report (France, 2019). In compliance with guidance document SANCO 5634/2009-rev.6.1 (European Commission, 2013), the RMS distributed the addendum to Member States, the applicant and the EFSA for comments on 12 November 2019. The RMS collated all comments in the format of a reporting table, which was submitted to EFSA on 11 March 2020. 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, France, and presents EFSA's scientific views and conclusions on the individual comments received.

1.2. Interpretation of the Terms of Reference

On 22 December 2014 the European Commission requested EFSA to provide scientific assistance with respect to the risk assessment of confirmatory data following approval of an active substance in accordance with Article 6(1) of Directive 91/414/EEC and Article 6(f) of Regulation (EC) No 1107/2009. EFSA's scientific views on the specific points raised during the commenting phase conducted with Member States, the applicant and EFSA on the risk assessment of confirmatory data for acibenzolar-S-methyl 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 11 April 2020.

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.

[1] 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.

[2] Commission Implementing Regulation (EU) 2016/389 of 17 March 2016 renewing the approval of the active substance acibenzolar-S-methyl 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 73, 18.3.2016, p. 77-80.

2. Assessment

The comments received on the pesticide risk assessment for the active substance acibenzolar-S-methyl 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. France, 2019. Addendum to the assessment report on acibenzolar-S-methyl, confirmatory data, November 2019. Available online: www.efsa.europa.eu.

2. France, 2020. Reporting table, comments on the pesticide risk assessment for acibenzolar-S-methyl in light of confirmatory data, March 2020.

References

ECHA (European Chemicals Agency) and EFSA (European Food Safety Authority) with the technical support of the Joint Research Centre (JRC), Andersson N, Arena M, Auteri D, Barmaz S, Grignard E, Kienzler A, Lepper P, Lostia AM, Munn S, Parra Morte JM, Pellizzato F, Tarazona J, Terron A and Van der Linden S, 2018. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. EFSA Journal 2018;16(6):5311,135 pp. https://doi.org/10.2903/j.efsa.2018.5311. ECHA-18-G-01-EN.

EFSA (European Food Safety Authority), 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2010;8(6):1637, 90 pp. doi:10.2903/j.efsa.2010.1637

EFSA (European Food Safety Authority), 2014. Peer review report to the conclusion regarding the peer review of the pesticide risk assessment of the active substance acibenzolar-S-methyl. EFSA Journal 2014;12(8):3691, 73 pp. doi:10.2903/j.efsa.2014.3691

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

Abbreviations

DAR	draft assessment report
DNT	developmental neurotoxicity
EATS	estrogen, androgen, thyroid, steroidogenic
EC	European Commission
ED	endocrine disrupting
EU	European Union
GD	guidance document
HCD	historical control data
MIE	molecular initiating event
MS	Member State
NIS	Na+/I- symporter
RAR	Renewal Assessment Report
RMS	rapporteur Member State
SD	standard deviation
T-modality	Thyroid-modality
TPO	Thyroid peroxidase

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

2. Effects on human and animal health

Reproductive toxicity				
No.	**Column 1** **Reference to addendum to assessment report**	**Column 2** **Comments from Member States / applicant / EFSA**	**Column 3** **Evaluation by rapporteur Member State**	**Column 4** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the RMS's assessment of confirmatory data**
2(1)	Vol. 1, 2.1.4, Conclusion of the assessment of T-modality	DE: Germany agrees with the assessment of the RMS.	RMS (March 2020): Noted. Thank you for your support. Addressed.	Addressed.
2(2)	Vol. 3, B.6.6.2, Developmental neurotoxicity study	AT: We agree to RMS' conclusion regarding the relevance of brain morphometric changes. We appreciate that the APPL's view as well as RMS final conclusion is included in the assessment. We are of the opinion that the APPL's argumentation is rather vague. Thus, we agree with the previous conclusion – it cannot be ruled out that the decrease of brain morphometric changes is not treatment-related. A connection to the increased acoustic startle	RMS (March 2020): Noted. Thank you for your support. Concerning HCD, please note that no new HCD have been provided in the context of confirmatory data process and the HCD available in the RAR were considered relevant during the peer review process (2013/2014). Furthermore, the new EFSA Administrative Guidance (2019) is not applicable as the dossier for confirmatory data was submitted by the applicant before its implementation.	Addressed.

		response might be plausible. This is supported by the fact that the values for dorsal cortex thickness are outside the provided HCD, the use of which is rather questionable, as the adjacent controls seem to be outside most of those. Additionally, for use of HCD the mean ±SD of all available individual values (all studies together), as well as min-max range and median should be reported (see Administrative Guidance, EFSA Supporting publication 2019:EN-1612).	Addressed.	

Neurotoxicity				
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(3)	General	EFSA: EFSA considers that the circumstantial evidence provided by the applicant are not enough to dismiss the treatment relationship and adversity of the morphometric findings observed in the cerebellum. EFSA agrees that the protocol procedure of longer fixation for the brain of animals belonging to the low and intermediate dose groups is adding uncertainty in the hazard characterization; however, the	RMS (March 2020): Noted. Thank you for your support regarding relevance of brain morphometric changes. Concerning ED properties, the RMS considered that further data should be provided regarding T-modality, as detailed in Volume 1: "*although the findings observed in the DNT study were not listed as "T-mediated" in the ED guidance but as "Sensitive to, but*	**Experts' consultation proposed.** EFSA recognise the need of an experts' consultation. Disruption of T-modality is a concern for DNT. However, it should be noted that additional, non-endocrine mediated effects, can induce DNT effects and the two issues, endocrine disruption and DNT, need an experts' discussion.

		effects observed in two linear morphometric evaluations of a major brain specific area can not be dismissed and are considered by EFSA as biologically relevant and adverse. In addition, a link to the transitory effect observed in the auditory startle reflex cannot be dismissed and should be considered in the weight of evidence. A mechanistic link with cell proliferation and migration is also plausible and investigations in this direction would have been informative. Although EFSA agrees that the results of the late-processed groups are potentially reflecting a processing artefact, the direct comparison of the control and high dose groups (which were processed simultaneously) indicate a treatment related effect and therefore the dose-response cannot be dismissed. EFSA also notes that a systematic literature review in line with the EFSA guidance was not provided. In conclusion, EFSA considers that the review provided by the applicant does not allow changing the conclusions on the peer review regarding the relevance of brain morphometric changes.	*not diagnostic of, T modality" and in order to address the confirmatory data requested by the Commission, the RMS considers that further data should be provided regarding the effects observed in the DNT study. It could be proposed to the applicant to investigate MIEs that could be responsible of such changes (e.g. NIS inhibition, thyroid receptor binding, deiodinase inhibition, TPO inhibition) and to measure thyroid hormones in the OECD TG 443, if conducted (see 2.2.4). Alternatively, US EPA thyroid assays (Guidance for Thyroid Assays in Pregnant Animals, Fetuses and Postnatal Animals, and Adult Animals, US EPA) could be relevant to address the data gap.*" In this reporting table, some MSs agree with the assessment of the RMS, whereas EFSA and the applicant are not of the same opinion. Therefore, in view of the different positions, it is proposed to discuss the ED properties of acibenzolar-S-methyl in an expert meeting. Recommendation for an expert meeting.	In addition, the assessment of the endocrine disrupting (ED) properties of acibenzolar-S-methyl and which additional test are needed to conclude on the ED properties can be subject of an experts' discussion. See also 2(6)-2(8) and 5(1). Additional note to further explain the EFSA opinion: EFSA/ECHA ED GD indicates that changes in thyroid hormones, even in the absence of thyroid histological change is a concern for the most sensitive population for DNT. In the available dataset there were no changes at thyroid histopathology; though TH and TSH were not measured (not in the data requirements). Current knowledge indicates that derangement of TH is relevant to DNT, particularly concerning neurological and cognitive impairments and auditory impairments. Morphological changes in the foetus/newborn are also reported in the AOP-wiki (hippocampal ectopia). There is however no indication on potential morphometric changes in the cerebellum. Therefore, at the experts' meeting, a detailed evaluation on the

		EFSA agrees that based on the available evidence provided in the dataset, the effect observed in the DNT study is unlikely consequent to endocrine toxicity		available evidence from the DNT study and information from other sources i.e. in vitro mechanistic studies, should be considered to properly address the DNT.

Further toxicological studies

No.	Column 1 **Reference to addendum to assessment report**	Column 2 **Comments from Member States / applicant / EFSA**	Column 3 **Evaluation by rapporteur Member State**	Column 4 **EFSA's scientific views on the specific points raised in the commenting phase conducted on the RMS's assessment of confirmatory data**
2(4)	Vol. 1, 2.2, ED assessment for EAS-modalities	DE: Germany agrees with the assessment of the RMS.	RMS (March 2020): Noted. Thank you for your support. Addressed.	Addressed.
2(5)	Vol. 1, 2.9, ED assessment and ED Excel file	AT: Please add the percentage values (to control) and/or incidences of the changes consistently under column "Observed effect (positive or negative)", where relevant. Additionally, please add the doses where no effect was observed. The way the ED lines of evidence tables are presented now in Vol. 1 as well as the Excel file it is quite difficult to assess and weigh the reported effects. What is meant with "slight" effect (e.g. skeletal anomalies rabbits #13 or food	RMS (March 2020): The RMS acknowledges the proposals of AT to improve the reporting of the observed effects in the Appendix E. Nevertheless, for the ED assessment of acibenzolar-S-methyl, these details on parameters "Sensitive to, but not diagnostic of EATS", target organ toxicity or systemic toxicity would not provide meaningful additional information, as no EATS-mediated adversity has been observed. It is noted that all effects (positive or	Addressed.

		consumption in dogs #4)? Where there any dose-relations? Only reporting the statistical significance may not be sufficient for a thorough evaluation. Hence, also for objective and transparent reporting, please consistently add the following information: 1- percentage values or some other quantification measure (as was done for a few studies, e.g. #8 inflammatory cell infiltration or #7 bodyweight) for all observed effects. 2- doses, also where no effects were observed.	negative) reported in the Excel file were checked by the RMS and are in line with the assessment provided in the DAR and in the RAR and agreed during the previous peer review (2014). If needed, study reports were also checked. Hence if a positive effect is reported in the Excel file, it means that it was considered adverse and treatment-related during the peer-review. Addressed.	
2(6)	Vol. 1, 2.9, ED assessment and ED Excel file	AT: We propose to discuss the ED assessment in an expert meeting. According to the ED assessment, the observed effects mostly concern parameters that are "sensitive to but not diagnostic of" EATS-mediated effects. This active substance might be the first one to affect SBND parameters only without any real EATS-mediated effects. Hence an expert meeting might be proposed. Apart from that, the ED assessment conducted by the RMS is acceptable. While EAS-mediated adversity was not sufficiently	RMS (March 2020): Noted. Thank you for your support. The RMS also recommends discussing the ED assessment in an expert meeting (please see 2(3)).	See experts' consultation proposal in 2(3).

		investigated and level 2 and 3 data need to be generated, we do agree that a T-mediated activity cannot be ruled out based on observed developmental effects (morphometric changes in the cerebellum and brain stem as well as an increased auditory startle). We also kindly note that spatial learning and memory was not completely assessed. However, according to the ED Guidance, in case T-mediated parameters are considered as sufficiently investigated, and no adversity was observed, the further conclusion would be to conclude that ED criteria are not met. Furthermore, we would like to highlight that RMS noted that for some studies the study reports were not available, and thus the organ weights and histopathological examinations could not be checked. Therefore we suggest to go with the scenario 2a(iii) – No adversity observed (not sufficiently investigated), no hormonal analysis, which would trigger further information.		
2(7)	Vol. 1, 2.9 Endocrine Disrupting Properties, p.82 2.3	Applicant: OECD456, OPPTS 890.1200 and OECD458 studies will be initiated to investigate A and S	RMS (March 2020): Noted. As mentioned in Volume 1, if all level 2 studies (OECD 456, OPPTS	See experts' consultation proposal in 2(3).

	Overall conclusion on the ED assessment for humans Conclusion on the assessment of EAS modalities	modalities. An OECD441 study will be conducted if triggered.	890.1200, OECD 458) are negative, level 3 study (OECD 441) should be provided. In case one of these studies is positive, level 5 study (i.e. OECD 443) should be performed. The RMS recommends discussing the ED assessment in an expert meeting (please see 2(3)) with the possibility to request further data to the applicant.	
2(8)	Vol. 1, 2.9 Endocrine Disrupting Properties, p.82 2.3 Overall conclusion on the ED assessment for humans Conclusion on the assessment of T modality	Applicant: Due to the absence of thyroid effects in any study with acibenzolar-S-methyl the generation of further in vivo data through the conduct of an OECD 443 or an EPA developmental thyroid study is not justified. If additional data are required to conclude on the thyroid modality MIEs that could be responsible for such changes would be investigated, and an updated review of relevant literature would be provided. A review of the available mammalian toxicology data for acibenzolar-S-methyl indicates that this compound occupies Scenario 1a for the thyroid modality as adequate data are available to conclude that this modality is not operant. Whilst the RMS has raised concerns around	RMS (March 2020): The RMS recommends discussing the ED assessment in an expert meeting (please see 2(3)). Please note that the position of the applicant on the results of the DNT study was fully included in the revised RAR. The RMS, as well as MSs and EFSA in this reporting table, considered that the review provided did not result in changing the conclusion of the peer review regarding the relevance of brain morphometric changes.	See experts' consultation proposal in 2(3).

	isolated changes in cerebellum parameters in a DNT study these changes are considered incidental to treatment, and the affected parameters are not considered "EATS-mediated" by the EFSA-ECHA ED guidance. The DNT study was performed in accordance with the only available testing guidance document available at the time. The EPA guidance document was newly issued at the time this study was performed. As such, laboratories were inexperienced with the testing guideline requirements therein. Compared with the more recent developmental neurotoxicity studies, the conduct of this study with acibenzolar-S-methyl was considered as a not yet technically proficient study, hence data were highly variable. Furthermore, there was limited concurrent historical control data available to provide sufficiently confident judgement on the strength of any changes and to set toxicity endpoints. One of the main challenges with the EPA guidance was that the neuropathological assessments would only be conducted for control		

	and high dose animals. Only if there was an effect of treatment observed at the high dose would the tissues from the low and intermediate dose animals be processed and examined. This has been highlighted by several authors as a deficiency following reviews of developmental neurotoxicity studies (Garman *et al.*, 2016, Tsuji, 2012, Raffaele *et al.*,2010, Bolon *et al.*, 2006) and it is considered that the then current stepwise approach to processing nervous tissue will introduce differences of variable degree in size and weight of brains and other tissues. The authors recommended that tissues from all groups be processed; this is now a requirement in the OECD guidance at the recommendation of the EPA. It is Syngenta's view that the decreased thickness of the cerebellar layers in the low and intermediate groups in this study were a symptom of extended storage in fixative compared with the control and high dose animals, and the tissues would deteriorate to a greater extent over time. Although most size reduction occurs during the initial stages of preservation, longer fixation does		

	cause more shrinkage (Garman *et al.*, 2016). Another concern is that histomorphometric measurement of the cerebellum can be problematic due to the highly-furrowed nature of the cortical surface and that relatively small differences in the cerebellar trimming plane can yield significantly different measures. When tissues for the low and intermediate dose tissues are being processed, the apparatus used to obtain the measurements will be recalibrated, thus potentially introducing another variable. It is because the intermediate dose tissues were not processed in the same manner as the control animals, that the statistically significant outcome for this group of animals is not considered reliable. The differences from control in the low and intermediate groups are therefore considered to be a technical artefact and unrelated to treatment with acibenzolar-S-methyl. The same finding in the high dose males is considered to be of no biological or toxicological significance due to the absence of any other treatment-related histopathological finding and a lack of corresponding behavioural deficits.		

	The weight of evidence strongly indicates that there are no findings in the package of repeat-dose in vivo studies, including the DNT study, that are associated with a thyroid disrupting mode of action. This conclusion can be further supported by additional investigation of MIE in vitro (e.g. NIS inhibition, thyroid receptor binding, deiodinase inhibition, TPO inhibition) to address concerns raised by the RMS. **References**: Bolon B. *et al.*, 2006. A 'Best Practices' Approach to Neuropathologic Assessment in *Developmental Neurotoxicity Assessment – for Today. Toxicologic Pathology*. **34:** 296-131 Garman R.H. et al, 2016. Recommended Methods for Brain Processing and Quantitative Analysis in Rodent Developmental Neurotoxicity Studies. *Toxicologic Pathology*. **44**(1): 14-42 Raffaele C.R. et al, 2010. The use of Developmental Neurotoxicity data in pesticide risk assessments. *Neurotoxicology and Teratology* **32**: 563-572		

		Tsuji R., 2012. Developmental Neurotoxicity guideline study: Issues with methodology, evaluation and regulation. *Congenital Anomalies* **52**: 122-128		

Other comments, incl comments on volume 4 (impurities, batches)				
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(9)	RAR	AT: The renewal assessment report currently contains new data only, while referring to the original dossier for points that did not change. While we are aware that RMS may not have had the time to collect all previous information from different addenda, the current RAR makes it highly difficult to evaluate the available information for assessing the endocrine disruption potential.	RMS (March 2020): The renewal assessment report was written in 2013 and the aim of the confirmatory data assessment was not to modify this RAR in order to include missing information, which are otherwise available in the DAR. Please note that both the DAR (1998) and the RAR (2014), the outcome of the peer review (2014), the available study reports, as well as ToxCast data, were checked by the RMS in order to gather all relevant information and include them in the Appendix E. Addressed.	Addressed.

5. Ecotoxicology

Other comments, incl available monitoring data				
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**
5(1)	Vol. 3, B.9.10 Endocrine disrupting properties	DE: A complete literature search according to EFSA/ECHA GD to be provided by the applicant.	RMS (March 2020): Indeed, the lack of literature search was highlighted by RMS (page 102). A literature search in line with the recommendations of EFSA/ECHA Guidance is required. In the absence of literature search together with relevant studies (see comments below), additional informations and studies might be requested, RMS then recommends discussing this point in an expert meeting with the possibility to request further data to the applicant. Recommendation for an expert meeting with the possibility to request further data.	**Peer Review proposed** to discuss the assessment of the endocrine disrupting (ED) properties of acibenzolar-S-methyl and which additional test are needed to conclude on the ED properties. See also 5(2), 5(3), 5(5), 5(6) and 2(3).
5(2)	Vol. 3, B.9.10 Endocrine disrupting properties	DE: Germany agrees with the assessment for T-modality for non-target organisms and the scenario 2a (iii) chosen by RMS. The given information/evidence is not sufficient to conclude either on T-mediated endocrine activity or on	RMS (March 2020): Noted. Thank you for your agreement. Addressed	See experts' consultation proposal in 5(1).

		the T-mediated adversity, thus further data need to be generated. We agree also with the proposed testing strategy (OECD TG 231 test should be provided).		
5(3)	Vol. 3, B.9.10 Endocrine disrupting properties	DE: Germany agrees with the assessment for the EAS-modality for non-target organisms and the scenario 2a (iii) chosen by RMS. The given information/evidence is not sufficient to conclude either on EAS-mediated endocrine activity or on the EAS-mediated adversity, thus further data need to be generated. We agree also with the proposed testing strategy (OECD TG 229, or in case of positive antagonistic action: OECD TG 234).	RMS (March 2020): Noted. Thank you for your agreement. Addressed	See experts' consultation proposal in 5(1).
5(4)	Vol. 1, 2.9 Endocrine Disrupting Properties p.85 3.1.4 Conclusion on the assessment of T modality	Applicant: No further comment	RMS (March 2020): Noted. Addressed	Noted.
5(5)	Vol. 1, 2.9 Endocrine Disrupting Properties p.91 3.2.4 Conclusion on the assessment of EAS modalities	Applicant: Syngenta agrees with the RMS that either an OECD 229 or OECD 234 could be appropriate to address EAS modality for non-target organisms, considering the available data for acibenzolar-s-methyl.	RMS (March 2020): RMS doesn't agree with the applicant's proposal. The RMS proposal to require an OECD 234 instead of an OECD 229 due to the potential anti-androgenic activity of the substance was previously agreed between MSs. RMS also notes that DE and EFSA agreed with the RMS proposal (comments 5(3) and 5(6)).	See experts' consultation proposal in 5(1).

	Syngenta does not, however, agree with the RMS that the OECD 229 would be insufficient to exclude anti-androgenic activity. Regarding detection of anti-androgenic activity, OECD TG 229 notes: *"a decrease in secondary sex characteristics in males should be interpreted with caution because of low statistical power and should be based on expert judgement and weight of evidence."* However, it is not clear whether this is equally true for all SSC endpoints, and all means of quantifying tubercle expression (count, prominence, score). There is in fact a significant weight of evidence in the peer-reviewed scientific literature that reduction in expression of nuptial tubercles is a reliable indicator of exposure to anti-androgenic substances. In a study of non-spawning adult fathead minnows (i.e. males and females housed separately), tubercle number was increased in	Moreover, in the absence of literature search together with relevant studies, additional informations and studies might be requested, RMS then recommends discussing this point in an expert meeting with the possibility to request further data to the applicant. Recommendation for an expert meeting with the possibility to request further data.	

	males and females exposed to dihydrotestosterone and decreased in male fish exposed to the pharmaceutical anti-androgen flutamide (Panter *et al.,* 2004). Ankley *et al*. (2004) reported competitive binding of flutamide and its hydroxylated metabolite to the cloned FHM androgen receptor and, in *in vivo* co-exposure studies that flutamide blocked the increase in tubercle score caused by exposure to trenbolone.		
	Of greater direct relevance to the OECD TG 229, Panter *et al*. (2012) reported reduction in tubercle prominence in spawning male fish exposed to the non-steroidal anti-androgen bicalutamide. Martinovic *et al*. (2008) exposed spawning pairs of FHM to vinclozolin for 21 d and reported concentration-dependent decrease in both tubercle score and fatpad index in male fish. In a separate study reported in the same paper, spawning groups of FHM were co-exposed to trenbolone and vinclozolin (200 and 700 μg/L) for 13 days. The trenbolone-induced increase in tubercle score in female fish was completely blocked at both concentrations of vinclozolin, while		

	the higher concentration reduced tubercle score in male fish with or without trenbolone co-exposure. Fecundity was also significantly reduced in females exposed to vinclozolin (Martinovic *et al*., 2008). Jensen *et al*. (2004) also reported significant reductions in female fecundity in breeding groups (2 male + 4 female, as per OECD TG 229) exposed to flutamide for 21 days. While tubercle score was not significantly affected, the pattern of effects on circulating hormone levels was highly comparable to that observed in studies with flutamide in rats, and the authors conclude that short-term (21-day) reproduction assays in the FHM are suitable for the detection of EDs, including anti-androgens. This conclusion is consistent with the findings of the US-EPA inter-laboratory validation of the fish short-term reproduction assay, which was run simultaneously across three independent contract laboratories (Biever *et al*., 2007). This exercise assessed the responsiveness of the FSTRA to a variety of chemicals representing different modes of action, and the results regarding secondary sexual		

characteristics are summarised in Table 1, below.

Chemical	Presumed MoA	Response of SSCs in males		
		Lab A	Lab B	Lab C
4-tert-octylphenol	estrogen	↓fatpad index	↓tubercle score and count	↓Tubercle score
Vinclozolin	Anti-androgen	↓Tubercle score	↓tubercle score fatpad weight, fatpad index	↓Tubercle score
Ketoconazole	Steroid synthesis inhi	no effect	↓tubercle score,	No effect

	bitor			
prochloraz	Steroid synthesis inhibitor and anti-androgen	↓tuber cle score	↓fatpad index	↓tuber cle score
SDS	Negative substance	no effect	no effect	↓tuber cle score fatpad weight, fatpad index

Table 1. Summary of responses of secondary sexual characteristics (SSC) endpoints to various reference EDs in the US EPA inter-laboratory validation of the FSTRA.

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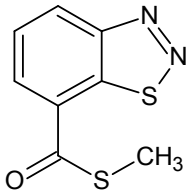
5(6)	Vol.1, section 3, Assessment of the ED properties on non-target organisms	EFSA: the ED assessment was provided in line with the ECHA/EFSA Guidance. The assessment is agreed as well as the additional studies that may be needed to further conclude on the ED potential of acibenzolar-S-methyl on non-target organisms	RMS (March 2020): Noted. Thank you for your agreement. Addressed	See experts' consultation proposal in 5(1).

Appendix B – Used compound codes

Code/trivial name[a]	IUPAC name/SMILES notation/InChiKey[b]	Structural formula[c]
acibenzolar-S-methyl	*S*-methyl benzo[1,2,3]thiadiazole-7-carbothioate O=C(SC)c1cccc2nnsc12	

(a): The metabolite name in bold is the name used in the conclusion.
(b): ACD/Name 2018.2.2 ACD/Labs 2018 Release (File version N50E41, Build 103230, 21 July 2018)
(c): ACD/ChemSketch 2018.2.2 ACD/Labs 2018 Release (File version C60H41, Build 106041, 7 December 2018)