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## Outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for 2,4-D 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 2,4-D are presented. The current report summarises the outcome of the consultation process organised by the rapporteur Member State Greece and presents EFSA's scientific views and conclusions on the individual comments received.

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**Keywords:** 2,4-D, peer review, confirmatory data, risk assessment, pesticide, herbicide**Requestor:** European Commission**Question number:** EFSA-Q-2021-000541**Correspondence:** pesticides.peerreview@efsa.europa.eu

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## Summary

The approval of 2,4-D was renewed in accordance with Regulation (EC) No 1107/2009 on 1 January 2016 by Commission Implementing Regulation (EU) 2015/2033. It was a specific provision of the approval that the applicant was required to submit to the European Commission, Member States and EFSA confirmatory information in the form of the submission of:

1. the complete results from the existing extended one-generation study by 4 June 2016; and
2. the Amphibian Metamorphosis Assay (AMA) (OECD (2009) Test No 231) by 4 December 2017

as to verify the potential endocrine properties of the substance.

In accordance with the specific provision, the applicant, the EU 2,4-D Taskforce (Adama, Dow AgroSciences and Nufarm), submitted an updated dossier in June 2016, which was evaluated by the designated rapporteur Member State (RMS), Greece, 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 9 December 2016. However, the reporting table was submitted by RMS to EFSA on 21 September 2021. Considering that new criteria to identify endocrine disrupters (Commission Regulation (EU) 2018/605) are applicable since 10 November 2018 and a guidance document is available, it is appropriate to consider these new criteria in the assessment of the confirmatory information requested. Therefore, the RMS was requested to re-assess the data according to the new criteria and EFSA/ECHA ED guidance of 2018 considering also the ED assessment provided in March 2022 by the applicant. The RMS distributed the revised addendum to Member States, the applicant and EFSA for comments on 11 November 2022. The RMS collated all comments in the format of a reporting table, which was submitted to EFSA on 24 February 2023. 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, Greece, and presents EFSA's scientific views and conclusions on the individual comments received regarding points 1 and 2.

In the area of toxicology, the assessment provided was considered comprehensive and in line with the EFSA/ECHA ED guidance of 2018. Overall, there is no evidence of a pattern of EAS and T mediated adversity in a sufficiently investigated dataset.

In the area of ecotoxicology, the submitted studies to address the confirmatory data requirement do not suggest a pattern of endocrine activity of 2,4-D for non-mammalian species. However, it is noted that too short study summaries of relevant literature studies were available. Although they do not seem to have an impact on the overall conclusion of the weight of evidence presented in line with the EFSA/ECHA ED guidance of 2018, it is suggested that extended study summaries are provided in the context of the renewal assessment for a proper evaluation.

Based on the available information it is considered that criteria as laid down in points 3.6.5 and 3.8.2 of Annex II to Regulation (EC) No 1107/2009, as amended by Commission Regulation (EU) 2018/605 are not met for both humans and non-target organisms.

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## 1. Introduction

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

The approval of 2,4-D was renewed in accordance with Regulation (EC) No 1107/2009<sup>1</sup> on 1 January 2016 by Commission Implementing Regulation (EU) 2015/2033<sup>2</sup>.

EFSA previously finalised a Conclusion on this active substance on 7 August 2014 in the EFSA Journal, that was updated in March 2017 (EFSA, 2014).

It was a specific provision of the approval that the applicant was required to submit to the European Commission, Member States and EFSA confirmatory information in the form of the submission of:

1. the complete results from the existing extended one-generation study by 4 June 2016; and
2. the Amphibian Metamorphosis Assay (AMA) (OECD (2009) Test No 231) by 4 December 2017

as to verify the potential endocrine properties of the substance.

In accordance with the specific provision, the applicant, the EU 2,4-D Taskforce (Adama, Dow AgroSciences and Nufarm), submitted an updated dossier in June 2016, which was evaluated by the designated rapporteur Member State (RMS), Greece, in the form of an addendum to the draft assessment report (Greece, 2016). 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 9 December 2016. However, the reporting table was submitted by RMS to EFSA on 21 September 2021. Considering that new criteria to identify endocrine disruptors (Commission Regulation (EU) 2018/605<sup>3</sup>) are applicable since 10 November 2018 and a guidance document is available, it is appropriate to consider these new criteria in the assessment of the confirmatory information requested. Therefore, the RMS was requested to re-assess the data according to the new criteria and EFSA/ECHA ED guidance of 2018 considering also the ED assessment provided in March 2022 by the applicant. The RMS distributed the revised addendum to Member States, the applicant and the EFSA for comments on 11 November 2022 (Greece, 2022). The RMS collated all comments in the format of a reporting table, which was submitted to EFSA on 24 February 2023 (Greece, 2023). 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, Greece, 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<sup>4</sup> 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 2,4-D are presented.

To this end, a technical report containing the finalised reporting table is being prepared by EFSA. This report should be finalised at the earliest convenience.

<sup>1</sup> 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.

<sup>2</sup> Commission Implementing Regulation (EU) 2015/2033 of 13 November 2015 renewing the approval of the active substance 2,4-D 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 298, 14.11.2015, p. 8-11.

<sup>3</sup> Commission Regulation (EU) 2018/605 of 19 April 2018 amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties. OJ L 101, 20.04.2018, p. 33-36.

<sup>4</sup> Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. OJ L 230, 19.08.1991, p. 1-32.

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

## 2. Assessment

The comments received on the pesticide risk assessment for the active substance 2,4-D 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. Greece, 2016. Addendum to the assessment report on 2,4-D, confirmatory data, December 2016. Available online: [www.efsa.europa.eu](http://www.efsa.europa.eu).
2. Greece, 2022. Revised addendum to the assessment report on 2,4-D, confirmatory data, 'Assessment of endocrine disrupting properties', November 2022 and revised in February 2023. Available online: [www.efsa.europa.eu](http://www.efsa.europa.eu).
3. Greece, 2023. Reporting table, comments on the pesticide risk assessment for 2,4-D in light of confirmatory data, February 2023.

### 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), 2014, updated in 2017. Conclusion on the peer review of the pesticide risk assessment of the active substance 2,4-D. EFSA Journal 2014;12(9):3812, 81pp. doi:10.2903/j.efsa.2014.3812
- 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

AMA	Amphibian Metamorphosis Assay
ANOVA	Analysis of variance
a.s.	active substance
BW	bodyweight
BWG	bodyweight gain
DNT	developmental neurotoxicity
EATS	estrogen, androgen, thyroid and steroidogenic
ED	Endocrine disruptor
EOGRTS	Extended One-Generation Reproductive Toxicity Study
FSTRA	Fish Short Term Reproduction Assay
GD	guidance document
GLP	good laboratory practices
LC <sub>50</sub>	lethal concentration, median
MEOGRT	Medaka Extended One Generation Reproduction Test
MS	Member State
MTC	Maximum Tolerated Concentration
MTD	Maximum Tolerated Dose
NTO	non-target organism
PND	post-natal day
RAR	Renewal assessment report
RMS	rapporteur Member State
mRNA	messenger ribonucleic acid
SVL	Snout-to-Vent Length
T3	triiodothyronine
T4	thyroxine
TK	toxicokinetics
TG	technical guideline
TSH	thyroid stimulating hormone
TSRC	threshold for saturation of renal clearance
VTG	vitellogenin
WoE	weight of evidence

## Appendix A – Collation of comments from Member States, applicant and EFSA on the pesticide risk assessment for the active substance 2,4-D in light of confirmatory data and the conclusions drawn by EFSA on the specific points raised

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

Not applicable.

### 2. Effects on human and animal health

Potential for endocrine disruption				
No.	Column 1 Reference to Assessment Report (vol., point, page)	Column 2 Comments from Member States, EFSA, notifier or public	Column 3 Evaluation by (RMS) rapporteur and - if available - (Co-RMS) Co-rapporteur / response from the notifier	Column 4 Data requirement or Open point (if data point not addressed or fulfilled)
1.	Addendum – Assessment of endocrine disrupting properties - Confirmatory Information  prepared to support the approval of the active substance according to Regulation (EC) 1107/2009, taking account of confirmatory data specified in Commission Implementing Regulation (EU) No 2015/2033.  Section 2.1.5	EFSA: agrees that scenario 1a should be applied and ED criteria are not met for thyroid modality for the active substance 2,4-D since there is no evidence of a T mediated pattern of adversity in a sufficiently investigated dataset.	RMS: Thank you.  Addressed.	Addressed.  The RMS provided a comprehensive assessment of the active substance including all available information and in line with the EFSA/ECHA ED guidance (2018). The assessment also includes a comparative evaluation versus the applicant proposal which was also based on the principles expressed in the EFSA/ECHA ED guidance (2018).
2.	Addendum – Assessment of endocrine disrupting properties -	EFSA: agrees that scenario 1a is applied and ED criteria are not met for EAS	RMS: Thank you.	Addressed.

Potential for endocrine disruption				
No.	Column 1 Reference to Assessment Report (vol., point, page)	Column 2 Comments from Member States, EFSA, notifier or public	Column 3 Evaluation by (RMS) rapporteur and - if available - (Co-RMS) Co-rapporteur / response from the notifier	Column 4 Data requirement or Open point (if data point not addressed or fulfilled)
	Confirmatory Information  prepared to support the approval of the active substance according to Regulation (EC) 1107/2009, taking account of confirmatory data specified in Commission Implementing Regulation (EU) No 2015/2033.  Section 2.2.5	modalities for the active substance 2,4- D since there is no evidence of an EAS- mediated pattern of adversity in a sufficiently investigated dataset.	Addressed.	The RMS provided a comprehensive assessment of the active substance including all available information and in line with the EFSA/ECHA ED guidance (2018). The assessment also includes a comparative evaluation versus the applicant proposal which was also based on the principles expressed in the EFSA/ECHA ED GD guidance (2018)
3.	Technical report: Outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for 2,4-D in light of confirmatory data	EFSA: the technical report contains parts of the LoEP that need to be updated following the confirmatory data and the updated ED assessment.	RMS: The technical should include updated information on ED assessment.  Addressed.	Addressed.
4.	Vol. 1, 2.1.5. Conclusion of the assessment T-modality, page 185	DE: We mostly support the RMS' and applicant's assessments on T-mediated modality, but additional elaboration should be made to strengthen their conclusion.  We agree that there were no substance-related patterns of thyroid adversity in mouse or dog.	RMS: Thank you for this comment.  The overall WoE suggests that a T- mediated pattern of adversity was not observed. In line with the EFSA/ECHA guidance on ED assessment, treatment- related adverse effects on endocrine organs observed at doses that overcome the MTD are not considered to be endocrine specific	Addressed.  The assessment performed by the RMS is considered fit for purpose and in line with the EFSA/ECHA ED guidance of 2018.

Potential for endocrine disruption				
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		<p>In rats, the most concerning observed thyroid effects were the modulation of thyroid hormones (decreased T4/T3 and increased TSH) and some pathological findings (smaller thyroid follicles with reduced colloid in 3/12 dams) in the highest dose group (40.2 mg/kg bw/d) at GD 17 in the EOGRTS (ID 19). Even though the changes in thyroid hormone levels at the highest dose were not statistically significant, they showed a dose-related pattern and thus were considered substance-related (see "Text Table 53" of the EOGRTS report). Nevertheless, looking at the hormone data of the individual dams (Figure 1 of the assessment), the negative outcomes of the DNT cohort of the EOGRTS and the lack of histopathological correlates for the thyroid hormone changes in F<sub>1</sub> pups, we are of the opinion that the thyroid findings in this EOGRT study are unlikely to be substance-related.</p> <p>One of key rationales from the RMS and applicant for concluding that the observed thyroid effects were not substance-related is that the thyroid effects were mainly observed at doses associated with systemic toxicity or</p>	<p>effects.</p> <p>Instead, systemic toxicity in these animals exceeds the capacity of the endocrine system to maintain a physiological homeostasis. As suggested in Table 5, page 174, no adverse effects on thyroid are observed at doses with no concomitant systemic effects.</p> <p>Moreover, thyroid adversity (changes in histology and thyroid hormone changes) was observed at doses where the MTD is exceeded (mortality, clinical signs, severe decreases in BW and BWG and liver and kidney toxicity) compromising the assessment of an endocrine specific adverse effect.</p> <p>In case of 2,4-D, exceedance of the MTD occurs due to disproportionate elevation of 2,4-D plasma concentrations, at doses above the threshold for renal clearance saturation (Saghir et al. 2013a).</p> <p>This point will be further elaborated in a revised assessment report for</p>	

Potential for endocrine disruption				
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		<p>above the “threshold for saturation of renal clearance” (TSRC). While this could be a plausible argumentation, the evidence to support this is not entirely sufficient.</p> <p>The RMS states in their conclusion: “Changes in thyroid hormones (and subsequent changes in thyroid weight) were observed only at doses above 45 mg/kg bw/day and always in the presence of systemic toxicity in terms of body weight, kidney and liver effects.” Could the RMS elaborate further on the degree of concomitantly observed systemic toxicity, please? What were the determinants/criteria for drawing the conclusion that thyroid effects in parallel with systemic toxicity or other target organ effects were not thyroid-mediated?</p> <p>Similarly, it would be highly beneficial for the review if the RMS or the applicant could elaborate here on the determination of the TSRC in rats and how this confounds or hinders the determination of thyroid disruption, e.g. why would the observed thyroid effects above the TSRC not be considered T-mediated? A reference of [REDACTED],</p>	<p>completeness.</p> <p>Open point. The RMS to include more information on the saturation of renal clearance of 2,4-D as provided in [REDACTED]. 2013a.</p>	

Potential for endocrine disruption				
No.	Column 1 Reference to Assessment Report (vol., point, page)	Column 2 Comments from Member States, EFSA, notifier or public	Column 3 Evaluation by (RMS) rapporteur and - if available - (Co-RMS) Co-rapporteur / response from the notifier	Column 4 Data requirement or Open point (if data point not addressed or fulfilled)
		2013 is provided in the assessment but no elaboration is given.		
5.	Vol. 1, 2.2.5. Conclusion of the assessment of EAS-modalities, page 451	<p>DE: We support the RMS' and applicant's assessments on the EAS-mediated modalities.</p> <p>The existing dataset from <i>in vitro</i> studies and prediction models (e.g., from ToxCast) unequivocally showed no indications of EAS-mediated activity.</p> <p>The EAS-related organ effects seen in repeated dose toxicity studies were observed concomitantly with systemic toxicity. It would be good to strengthen the conclusion to assess and discuss if systemic toxicity already occurred prior to the observed EAS-related effects.</p> <p>Similar to our comment on T-mediated modality, please give some further elaboration on the determination of TSRC and how this confounds the determination of EAS-mediated effects as this is one of the main argumentations for concluding that the EAS-related effects are not endocrine mediated.</p> <p>Lastly, the RMS mentions "Paraovarian</p>	<p>RMS: Thank you for this comment.</p> <p>Details on the assessment if EAS-mediated adversity in the rat in relation to systemic toxicity are tabulated in the RAR (Assessment and conclusion by RMS).</p> <p>Further information on the saturation of renal clearance of 2,4-D (██████████ 2013a) will be added in the revised assessment report.</p> <p>Regarding paraovarian cysts, further information from the public literature will be included in the revised assessment report.</p> <p>Open point.</p> <p>The RMS to include more information on the saturation of renal clearance of 2,4-D as provided in ██████████. 2013a.</p> <p>The RMS to include more information from public literature on the relevance of paraovarian cysts in a revised assessment</p>	<p>Addressed.</p> <p>The assessment performed by the RMS is considered fit for purpose and in line with the EFSA/ECHA ED GD.</p> <p>Open point addressed.</p>

Potential for endocrine disruption				
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		cysts are not considered endocrine-related as they are considered to arise from vestigial remnants of the mesonephric and paramesonephric (Müllerian) ducts in line with ATP Atlas." Please provide references and further elaboration on this statement (while EAS-mediated modalities might not be involved, non-EAS-mediated mechanisms might be).	report.	
6.	Page 11, Section 2 ED assessments for humans	APPL: The applicant considers TK data and excessive exposure following saturation of renal clearance and high dose levels is a key contextual consideration in interpreting the potential human relevance of 2,4-D toxicity findings and should be considered when performing a weight of evidence assessment. The applicant suggests including data supporting the concept of the threshold for saturation of renal clearance (TSRC) into the assessment.	RMS: The publication by [REDACTED] (2013a) regarding saturation of renal clearance of 2,4-D will be further elaborated in a revised assessment report.  Open point.  See open points 4 and 5	Addressed.  The TSRC is contextualized in the assessment provided by the RMS. The WoE includes the relationship between the TSRC, the MTD and the endocrine endpoints.
7.	Page 190, Assessment of thyroid adversity in the rat, last bullet point.	APPL: The applicant notes that an increase in thyroid weight was observed in study ID 13 at 15 mg/kg bw/day, without effect on T4 levels or thyroid histopathology. In addition, kidney histopathology was observed at this dose level (brown pigment present in	RMS: Noted and agreed.  This observation further supports that thyroid effects after of 2,4-D exposure in rats are non-specific consequences of toxicity and not observed in the absence of	Addressed.

Potential for endocrine disruption				
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		tubular cell (M+F) at 12 and 24 months. Pelvic microcalculi (M) at 24 months).	systemic toxicity.  Addressed	
8.	Page 190, Assessment of thyroid changes in the sensitive population (EOGRT study), second bullet point, second line.	APPL: The applicant notes that exposure in F1 male offspring was 76.6 mg/kg bw/day.	RMS: The value of 76.6 mg/kg bw/day corresponds to Set 1a Males (PND 28-69) of the 800 ppm group. There are not compound intake data available from PND21 to 28. So, the RMS considers it more appropriate to use the value of 45 mg/kg bw/day for compound intake at PND22 which is derived from parental animals of the same dose group.  Addressed.	Addressed.
9.	Page 192, Overall assessment of thyroid, last paragraph	APPL: The applicant agrees with the overall mammalian T modality conclusion that ' <i>scenario 1a is applied and ED criteria are not met for thyroid modality for the active substance 2,4-D.</i> '	RMS: Noted. Thank you.  Addressed.	Addressed.  T criteria not met.
10.	Page 458, Appendix E data and Lines of Evidence, General comment, first paragraph	APPL: The applicant agrees for adult rats that effects on absolute testes weight are not considered secondary to decreased body weight, however testes in weanling/juvenile rats (Carney et al., 2004, Rehm et al., 2008) and dogs (Creasy, 2003 and Goedken et al., 2008)	RMS: Noted. Thank you.  Addressed.	Addressed.  The effect on testicular weight was noted as potentially dependent on body weight in the specific population

Potential for endocrine disruption				
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		<p>have been demonstrated to be different to adults and are impacted by delayed growth / development which may be indicated by decreased body weight.</p> <p>Carney EW, Zablony CL, Marty MS, Crissman J, Anderson P, Woolhiser M, Holsapple M (2004). The effects of feed restriction during in utero and postnatal development in rats. Toxicol Sci. 82:237–249.</p> <p>Creasy D (2003). Evaluation of Testicular Toxicology: A Synopsis And Discussion Of The Recommendations Proposed by the Society of Toxicologic Pathology. Birth Defects Research (Part B) 68:408–415.</p> <p>Goedken MJ, Kerlin RL, Morton D (2008). Spontaneous and Age-Related Testicular Findings in Beagle Dogs. Toxicologic Pathology, 36: 465-471</p> <p>Rehm S, White TE, Zahalka EA, Stanislaus DJ, Boyce R, Wier PJ (2008). Effects of Food Restriction on Testis and Accessory Sex Glands in Maturing Rats. Toxicologic Pathology, 36: 687-694, 2008</p>		(weanling/juvenile rats).

Potential for endocrine disruption				
No.	Column 1 Reference to Assessment Report (vol., point, page)	Column 2 Comments from Member States, EFSA, notifier or public	Column 3 Evaluation by (RMS) rapporteur and - if available - (Co-RMS) Co-rapporteur / response from the notifier	Column 4 Data requirement or Open point (if data point not addressed or fulfilled)
11.	Page 458, Assessment of EAS-mediated adversity in the rat, dose concordance table	APPL: The applicant notes that for Study ID: 4 some measured parameters have not been included in the table.  For Study ID: 4: Ovary, uterus, testes and prostate histopathology was assessed and no effect noted at all doses.	RMS: According to the study report of ID4 (Serota, Project no.: 2184-102) no histopathological evaluation has been performed for the dose groups of 1, 5 and 15 mg/kg bw/day. Histopathology of the ovary, uterus, testes and prostate has been assessed only in the control and highest dose groups.  Addressed.	Addressed.
12.	Page 471, Assessment of EAS-mediated adversity in the rat, findings from the EOGRT study, 1 <sup>st</sup> bullet	APPL: The applicant notes that at PND22 terminal body weights were significantly decreased by -10% in male weanlings at 9.2 and 76.6 mg/kg bw/day (both P<0.05) and by -9% at 28.4 mg/kg bw/day (not statistically significant). A previous feed restriction study has established that weanling organ weights, including testes, are subject to change with alterations in body weight and delayed growth (Carney et al., 2004).  Carney EW, Zablony CL, Marty MS, Crissman J, Anderson P, Woolhiser M, Holsapple M (2004). The effects of feed restriction during in utero and postnatal development in rats. Toxicol Sci.	RMS: Noted. Data are already included in the RMS assessment. There is no dose-response effect on the absolute testis weight in the EOGRT study.  Addressed.	Addressed.

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		82:237–249.		
13.	Page 471, Assessment of EAS-mediated adversity in the rat, findings from the EOGRT study, 2 <sup>nd</sup> bullet	<p>APPL: The applicant notes that slight delay in age of preputial separation are consistent with Marty et al. (2003), who reported that a similar body weight decrement (10%) in juvenile male rats delays puberty up to 1.8-days. Therefore, the applicant considers this change to related to delayed growth, and not direct ED-related activity.</p> <p>Marty MS, Johnson KA, Carney EM (2003). Effect of Feed Restriction on Hershberger and Pubertal Male Assay Endpoints. Birth Defects Research (Part B) 68:363–374.</p>	<p>RMS: Noted and agreed. This information is already commented in the RMS assessment.</p> <p>Addressed.</p>	<p>Addressed.</p> <p>The relationship between preputial separation and changes in body weight in the juvenile male rat population is correctly addressed in the RMS assessment.</p>
14.	Page 471, Assessment of EAS adversity in dog, 2nd bullet	<p>APPL: The applicant notes in Study ID: 7, 8, 25 lower absolute testes weight and higher incidence of hypospermatogenesis and giant cells and inactive/juvenile prostate has been observed.</p> <p>In published literature, immature dogs (less than 9 months of age) have been reported to have control incidences as high as 75% of both decreased testes weight and hypospermia (Goedken et al. 2008). In Goedken et al. (2008),</p>	<p>RMS: Noted and agreed. This information is already commented in the RMS assessment.</p> <p>Effects in testes weight and spermatogenesis in dogs were only observed in the presence of severe systemic toxicity.</p> <p>Addressed.</p>	<p>Addressed.</p> <p>The assessment of testicular changes is appropriate in the context of study ID 7 where the assessment of immature animals is confounded by systemic effect.</p>

Potential for endocrine disruption				
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		<p>atrophic/hypoplastic tubules in the testes were seen in 26.3% of all dogs, with 25–40% of dogs under 12 months old showing this finding. The reported age of dogs in studies ID 7, 8, 25 were 4–6 months old at study initiation, a review of body weight data suggests many of the dogs were on the low end of this age range (Charles et al., 1996). Dogs were therefore juvenile (7–9 months old) at terminal sacrifice. This observation supports the conclusion that the dogs used in the studies were immature. The applicant considers that the decreased testes weights and histopathological findings are an artefact related to the young age of these animals during study, in addition to the delayed development caused by the excessive systemic toxicity, and not direct ED-related activity.</p> <p>Supporting this conclusion a chronic study in dogs (ID: 9), where animals were older at termination, showed no exposure-related effects on testes weights or histopathology following a one-year exposure to 2,4-D at a high dose level (10 reduced to 7.5 mg/kg/day).</p>		

Potential for endocrine disruption				
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		Charles J, Dalgard D, Cunny H, Wilson R, Bus J (1996). Comparative subchronic and chronic dietary toxicity studies on 2,4-dichlorophenoxyacetic acid, amine, and ester in the dog. Fundam Appl Toxicol. 29:78–85 (2,4-D 2-EHE reported under study ID: 25 and pure 2,4-D data reported under study ID: 8 and 9).  Goedken MJ, Kerlin RL, Morton D (2008). Spontaneous and Age-Related Testicular Findings in Beagle Dogs. Toxicologic Pathology, 36: 465-471.		
15.	Page 474 and 475, Table of submitted publications and open literature Lemaire et al, (2006) (Study ID: 73) and Kojima et al, (2004), Study ID 67.	APPL: The applicant would like to note that antagonism was not assessed via competitive [ <sup>3</sup> H]-E2 uptake assay, but via a competitive decrease in transactivation measured by luminescence.	RMS: Thank you for pointing this out. Description of test systems used in the studies by Lemaire (2006) and Kojima (2004) will be corrected in a revised assessment report.  Addressed.	Addressed.
16.	Page 477, Overall assessment of EAS modalities, last paragraph	APPL: The applicant agrees with the overall mammalian EAS modalities conclusion that <i>'scenario 1a is applied and ED criteria are not met for EAS modalities for the active substance 2,4-D since there is no evidence of EAS-mediated</i>	RMS: Thank you.  Addressed.	Addressed.  Criteria for EAS-modalities are not met.

Potential for endocrine disruption				
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		<i>adversity.</i>		
17.	<p>Page 192, Overall assessment of thyroid, 3<sup>rd</sup> bullet point</p> <p>Page 477, Overall assessment of EAS modalities, 3<sup>rd</sup> bullet point</p> <p>Page 478, Section 2.3.Overall conclusion on the ED assessment for humans, Assessment and conclusion by the RMS, both paragraphs</p>	<p>APPL: The applicant notes that the RMS considers '<i>2,4 D is a well-known nephrotoxin through accumulation in renal proximal tubules</i>'. The applicant agrees that 2,4-D is a known nephrotoxin at high doses. However, the applicant would like to comment that saturation of the renal clearance does not necessary lead to histopathology changes and nephrotoxicity.</p>	<p>RMS: Noted. You may refer to response to comments 4, 5 and 6.</p> <p>Open point.</p> <p>See open points 4, 5 and 6.</p>	<p>Addressed.</p> <p>The consequences of nephrotoxicity and of the saturation of the renal clearance on the assessment of the ED properties is adequately contextualized in the assessment.</p>
18.	Various typographical errors	<p>APPL: The applicant suggests there are a small number of typographical errors.</p> <p>Page 4, 4<sup>th</sup> paragraph, 9<sup>th</sup> line: A full stop is missing between assessment Criteria</p> <p>Page 164, Table 3, LoE, Study ID: 86, Mortality, effect description is wrong – change effect to increase</p> <p>Page 166 to 185: headers and footers need to be removed as refer to the applicants assessment</p> <p>Page 174, 3<sup>rd</sup> paragraph, 1<sup>st</sup> line: Add a link to the dose concordance table, Table 5</p> <p>Page 188, Assessment and conclusion by</p>	<p>RMS: Thank you for pointing out these errors. All typos will be corrected in a revised assessment report.</p> <p>Addressed.</p>	Addressed.

Potential for endocrine disruption				
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		<p>the applicant, 3<sup>rd</sup> line: Add a link to the dose concordance table, Table 5</p> <p>Page 189, Table of studies- Study ID 7, this was conducted to OECD TG 409 (not 408)</p> <p>Page 189, Table of studies- Study ID 9, this was conducted to OECD TG 452 (not 409)</p> <p>Page 189, Table of studies- Add Study ID: 10, 28 day rat inhalation OECD TG 412, thyroid histopathology assessed.</p> <p>Page 189, Assessment of thyroid adversity in the rat, 1<sup>st</sup> line: No changes in thyroid histopathology were observed in <b>6</b> studies (including <b>one</b> carcinogenicity studies) (not 8 studies and 2 carcinogenicity studies).</p> <p>Page 190, Assessment of thyroid adversity in the rat, last line: list of studies IDs should include <b>79</b>, not 19</p> <p>Page 190, Assessment of thyroid changes in the sensitive population (EOGRT study), first row, the changes in hormones levels are the wrong way round - change to <b>T4</b> and <b>T3</b> (8.7% and 6.8% respectively).</p> <p>Page 191, Assessment of thyroid adversity in mouse, 1<sup>st</sup> bullet, 1<sup>st</sup> line: (including <b>two</b> 104-week carcinogenicity <b>studies</b>)</p>		

Potential for endocrine disruption				
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		<p>Page 437, Table 9, LoE, Study ID: 86, Mortality, effect description is wrong – change effect to increase</p> <p>Page 446, Table 11, 3<sup>rd</sup> row, first point, last sentence - as an antagonist or agonist and not inhibition of aromatase activity.</p> <p>Page 472, 1<sup>st</sup> row: ALT (+51%)</p> <p>Page 474, Petit et al, (1997) Study ID 77: Purity was not reported.</p> <p>Page 475, Sun et al, (2012) Study ID 64. Maximum tested concentration was 3 mg/L, <math>1.3 \times 10^{-5}</math>.</p> <p>Page 475, Vonier et al, (1996) Study ID 71: No effect on ER binding (not (anti-)estrogenic activity)</p>		

### 3. Residue data

Not applicable.

### 4. Environmental fate and behaviour

Not applicable.

## 5. Ecotoxicology

Potential for endocrine disruption				
No.	Column 1 Reference to Assessment Report (vol., point, page)	Column 2 Comments from Member States, EFSA, notifier or public	Column 3 Evaluation by (RMS) rapporteur and - if available - (Co-RMS) Co-rapporteur / response from the notifier	Column 4 Data requirement or Open point (if data point not addressed or fulfilled)
1.	Section 3, ED assessment wild mammals	EFSA: for wild mammals, the conclusion and comments related to mammalian studies apply.	RMS: The conclusion was amended with the phrase "mammalian toxicology studies".  Addressed.	Addressed.  The addendum has been amended by clarifying the conclusion for wild mammals.
2.	Section 3, addendum ED assessment NTOs	EFSA: the assessment is well presented. However, summaries of the studies are not presented. In the previous version of the addendum only summary of the AMA is available. Therefore, it is recommended that a compiled version is produced where study summaries and ED assessment are presented all together. The way the document is presented do not allow a proper peer review. For example, fish studies and literature studies are not presented either in this version or the previous one. For the literature studies, it is also recommended that relevance and reliability criteria are defined and applied consistently to the available studies.	RMS: The summaries presented in the RAR have been added in the addendum.  For literature studies that the summary is missing, the applicant is requested to provide it, applying the relevance and reliability criteria as well.  <b>Applicant to provide the missing summaries of several literature studies, applying the relevance and reliability criteria as well</b>  (please refer to the 2,4-D_Draft Addendum confirmatory information).	Addressed.  According to Commission Implementing Regulation (EU) 2015/2033, an Amphibian Metamorphosis Assay according to OECD TG 231 was provided and did not show a pattern of T-mediated endocrine activity. Moreover, a weight of evidence in line with the ECHA/EFSA ED guidance was also available. A number of literature studies were also retrieved. Based on the short summaries available, it seems those papers do not have an impact on the overall conclusion. Nevertheless, it is recommended to submit extended study summaries allowing an independent evaluation and to better contextualize the results

Potential for endocrine disruption				
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				of the literature studies in the ED assessment for the renewal assessment.
3.	Section 3, excel file ED studies	EFSA: the RMS is mentioning the excel file. However, it seems this is not available to EFSA and could not be checked.	RMS: The excel file was not uploaded by mistake. The mistake will be corrected in this upload.  Addressed.	Addressed.  The Excel file (Appendix E of the ECHA/EFSA ED guidance) was provided.
4.	Section 3, Padilla et al., 2012	EFSA: It is noted that the study was considered to ' <i>provide only supportive data for the lack of ED-related adversity, since the study provided little information concerning potential ED-related effects</i> '. However, the study is a screening for developmental toxicity and a such should not be considered relevant for its use in the ED assessment.	RMS: We agree with the comment. This developmental screening assay provides only supportive data for the the lack of ED-related adversity as an open-literature publication study. To have the EAS-mediated endocrine activity for non-target organisms considered to be sufficiently investigated, A FSTRA study was provided. The summary was included in the Addendum (study ID:30).  Addressed.	Addressed.  A summary was included in the addendum.  See also reply to comment 2.
5.	Section 3, Crago (2015)	EFSA: It is stated that: ' <i>Moreover, it should be noted that █████ (2010) [ID: 30] provides a more robust assessment of vitellogenin since the study was conducted according to OECD TG 229, was GLP compliant and both sexes were tested at higher concentrations for a longer period</i>	RMS: The statement was revised.  Addressed.	Addressed.  The addendum was updated by clarifying the reliability assessment.

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		<p><i>of time over critical life stages. In addition, in █████ (2010) blood plasma vitellogenin levels were assessed using a validated methodology, whereas in █████ (2015) hepatic mRNA vitellogenin levels were assessed which may not accurately reflect actual blood levels in the fish'.</i></p> <p>Although it is agreed that gene expression in isolation are not an indication of an endocrine modulation and it is also agreed that the data from █████ (2010) do not show any positive evidence of endocrine activity, it is not appropriate to say that the results in █████ (2010) are more reliable simply because the study is GLP. GLP should not be considered as a criterion for reliability. The 2 studies should be assessed for reliability applying defined reliability criteria. After, the findings in both studies should be contextualised in the weight of evidence. It is therefore proposed to slightly revisit the statement</p>		See also reply to comment 2.
6.	Section 3, Orton et al. 2009	EFSA: As indicated in the comment above a study summary and a full evaluation of the study should be provided for all the assessed studies included in the ED assessment. For example, in the addendum, it is not clear why Orton et al is only considered supportive for ED	<p>RMS: Please refer to comment 2.</p> <p>The study summary was included in the addendum. Although the study presents some deficiencies, the observations support the view that there is no evidence of endocrine activity.</p>	See reply to comment 2.

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		activity and this should be further substantiated.	Addressed.	
7.	Section 3, Lenkowski, J.R., 2010 (ID:76)	EFSA: As indicated in the comment above a study summary and a full evaluation of the study should be provided for all the assessed studies included in the ED assessment. Are the observations in this study considered relevant for ED assessment considering that only edemas and intestine abnormalities were assessed? Moreover, there is no mention that the concentrations in the study were not measured and this is considered a key reliability criterion.	RMS: The applicant is requested to provide the summary of the aforementioned study including the relevance of the observations regarding endocrine activity.	See reply to comment 2.
8.	Section 3, Raldua and Babin, 2009.	EFSA: As indicated in the comment above a study summary and a full evaluation of the study should be provided for all the assessed studies included in the ED assessment. It is not clear if the study is considered reliable by the RMS. It is noted that from the paper it is not very clear at which concentration decrease in T4 immunoreactivity was observed.	RMS: The applicant is requested to provide a more detailed summary of the aforementioned study.  The study is considered as supportive information since a single dose was tested and the group size was relatively small.  The decrease in T4 immunoreactivity was observed at the single concentration tested.  Addressed.	See reply to comment 2.

Potential for endocrine disruption				
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9.	Table 1, addendum	EFSA: in the table of the dataset considered in the assessment, the literature studies are listed at the end without differentiating whether the study is more related to mammalian toxicology or ecotoxicology. Therefore, it is unclear which studies have been used in the 2 assessments.	RMS: The literature studies have been divided in two different lists: Literature (Mammalian toxicology) and Literature (Ecotoxicology).  Addressed.	Addressed.  The addendum was updated by dividing the papers relevant for mammalian toxicology and ecotoxicology, respectively.
10.	Lines of Evidence T-modality and study 70 and 84	EFSA: In the table with the lines of evidence a number of parameters are listed which are from studies not even described in the text. It is clear those are in vitro studies with mammalian cells. However, it would be good to also refer to those in the text. For example, it is noted that study 70 and 84 are only mentioned in the tables with the lines of evidence. Moreover, in the ecotox section, it is mentioned that study 70 is considered of low quality. However, it should be clarified what 'low quality' means and what have been the criteria to reach that conclusion. It is not clear whether study 84 is reliable and this should be clarified.	RMS: Please take into consideration that in building the lines of evidence, apart from the EATS-mediated parameters as well as the parameters "non sensitive to, but diagnostic of", the in vitro mechanistic results are included in the analysis assessment.  These in vitro mechanistic results are withdrawn from the mammalian toxicology section. The deficiencies of both these studies (ID:70 and ID:84) are already reported in the grey box " <b>Assessment and conclusion by RMS</b> " at the end of 3.1 Section.  Both these studies are considered unreliable. For more details please refer to the Mammalia Toxicology Section.  Addressed.	Addressed.  The way the lines of evidence have been built was clarified in column 3.

Potential for endocrine disruption				
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11.	Section 3.2.1, EAS-modalities	EFSA: It is not clear why in vivo mechanistic parameters measured in the OECD TG 240 were listed. Please, note that sex ratio is an EAS-mediated parameter and not an in vivo mechanistic parameter.	<p>RMS: In vivo mechanistic parameters measured in the OECD TG 240 were reported in order to demonstrate that the EAS-mediated adversity for non-target organisms was not sufficiently investigated. Moreover, it is reported that: The 'in vivo mechanistic' and 'EAS-mediated' parameters measured in the MEOGRT (OECD TG 240) are:</p> <ul style="list-style-type: none"> <li>• Vitellogenin (VTG) (in males and females),</li> <li>• Sex ratio (female and male biased).</li> </ul> <p>Thus, vitellogenin refers to 'in vivo mechanistic' and sex ratio refers to 'EAS-mediated', respectively.</p> <p>Addressed.</p>	<p>Addressed</p> <p>The information reported in the addendum was clarified in column 3 in relation to the EAS-mediated parameters investigated in the OECD TG 240.</p>
12.	Section 3.2.1. ED assessment for EAS-modalities	EFSA: As indicated in the comment above study summaries and a full evaluation of the studies included in the assessment should be provided for all the assessed studies included in the ED assessment. This is the case for study 71, 77, 81 ad 82.	<p>RMS: Please refer to comment 2.</p> <p>The summaries presented in the RAR have been added in the addendum.</p> <p>For literature studies that the summary is missing, the applicant is requested to provide</p>	See reply to comment 2.

Potential for endocrine disruption				
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			it, applying the relevance and reliability criteria as well.  <b>Applicant to provide the missing summaries of several literature studies, applying the relevance and reliability criteria as well</b>  (please refer to the 2,4-D_Draft Addendum confirmatory information).	
13.	Section 3.2.1 Study 32	EFSA: a field study with common vole was included in the assessment for EAS-modalities. However, it is not clear why this study was considered relevant considering it is a field study and therefore not really appropriate for hazard identification.	RMS: The study was included in the assessment only for clarity reasons.  It is considered to be only supportive data for the lack of ED-related adversity, since provides negligible information concerning potential ED-related effects.  Addressed.	Addressed.  As reported in column 2, the field study with common vole should be reconsidered in the context of the weight of evidence for ED assessment for the renewal assessment. However, it is agreed that this study provides little information on hazard identification.
14.	Table 23	EFSA: In table 23, scenario 1b was erroneously selected. It should be 2a(ii)	RMS: Comment acknowledged. The mistake was corrected.  Addressed.	Addressed.  Table 23 of the addendum was corrected according to the comment in column 2.

Potential for endocrine disruption				
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15.	Add. Conf. Information, Assessment of endocrine disrupting properties 3.1.5. Conclusion on the ED assessment for T-modality	<p>DE: For the AMA of [REDACTED] (2010) there is no information whether the MTD/MTC was reached or not. Please, add the information on this very important point and discuss the implications for the reliability of the test.</p> <p>Moreover, we rechecked the test and noted that there is a concentration-dependent increased for snout-vent length (alongside wet weight) for 21 days (please refer to Table B.9.11/01-3 of the Addendum – Confirmatory Information from 2016). We want to ask whether this is relevant and further discussion is needed. It was non-significant and non-monotonic, but the group which was the reason for non-monotonicity is the group with the dead animal (group could be excluded also from significance testing) and non-significance might be questioned if the MTC was not reached.</p> <p>As side note, the statistics of [REDACTED] (2010) seem to be from an older guideline with conducting an ANOVA only. We do not expect a different outcome with current statistics (tests for normality, variance homogeneity and presumably Dunnett test as post hoc test), but this could be checked, too.</p> <p>Lastly, please note further that in case of a negative AMA the scenario 1a is</p>	<p>RMS: Since no effects were observed for any of the parameters tested, the MTC, according to OECD 231, is defined as the highest test concentration of the test item which results in less than 10 % acute mortality or as 1/3 of the acute LC50 value from other aquatic species (i.e. fish).</p> <p>According to EFSA conclusion of 2.4-D (2014), the lowest LC50 value for fish is 100 mg/L.</p> <p>Thus, the MTC could be set at</p> <ul style="list-style-type: none"> <li>33.3 mg/L (1/3 of the acute LC50).</li> </ul> <p>In the study, concentrations of 0.273, 3.24, 38.0, 113 mg/L (mean measured) were used. Consequently, it can be considered that the MTC was reached.</p> <p>Since the MTC was reached and the concentration-dependent increase for snout-vent length for 21 days was not significant, it is not considered to be relevant. There is no effect for snout-vent length.</p> <p>Regarding the AMA scenario selected, we agree that since a negative AMA test is available, adversity (apart from activity) is</p>	<p>Addressed.</p> <p>The information on the Maximum Tolerated Concentration (MTC) in the study by Coady et al., (2010) (AMA) was added in the addendum.</p> <p>Please note that Snout-to-Vent Length (SVL) is a 'sensitive to but not diagnostic of T' parameter. Therefore, in isolation, it does not raise any concern on a possible activity through the T-modality.</p>

Potential for endocrine disruption				
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		appropriate. As there were literature data which could suggest activity (and are overwritten by the negative AMA) we would suggest using this scenario.	considered to be sufficiently investigated as well. Thus, scenario 1a applies.  Addressed.  The MTC justification was added in the grey box at the end of Section 3.1	
16.	Add. Conf. Information, Assessment of endocrine disrupting properties 3.2.5. Conclusion on the ED assessment for EAS-modality	DE: Also, for the study of [REDACTED] (2010) there is no information whether the MTD/MTC was reached or not. Please, add the information on this important point and discuss the implications for the reliability of the test. However, as e.g. there was 17% mortality in the highest test group in one replicate reaching the MTC seems not unlikely.	RMS: Since in Study ID 30, fecundity was significantly decreased in the high treatment (96.5 mg/L) group as well as 17% mortality was observed in this highest concentration, it could be considered that different effects were observed in the study, thus, the MTC (maximum tolerated concentration) was reached.  Addressed. The MTC justification was added in the grey box at the end of Section 3.2 for clarity reasons.	Addressed.  The addendum was revised including information on the MTC in the study by [REDACTED] (2010) (Fish Short Term Reproduction Assay (FSTRA)).
17.	Page 487, ED assessment for T-modality, and Page 508, ED assessment for EAS-modality	APPL: The applicant notes that the RMS assessment says that ' <i>Some minor comments are presented below the Lines of Evidence table</i> '. However the applicant could not find these comments.	RMS: Comment acknowledged. The phrase was corrected.  Addressed.	Addressed.  The addendum was clarified following the comment in column 2.
18.	Page 488, Overall assessment of thyroid, last	APPL: The applicant agrees with the overall NTO T modality conclusion that ' <i>scenario 2a (ii) is applied and ED criteria are not</i>	RMS: Comment acknowledged.	Addressed.

Potential for endocrine disruption				
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	paragraph	<i>met for thyroid modality (activity and adversity) for the active substance 2,4-D.</i>	However, since a negative AMA test is available, adversity is considered to be sufficiently investigated as well.  Thus, scenario 1a is applied.  Addressed.	The addendum was updated by selecting the appropriate scenario in accordance with the ECHA/EFSA ED guidance (2018).
19.	Page 507, Table 23: Selection of relevant scenario	APPL: The applicant considers that, and in agreement with the RMS, as described in the conclusion on the ED assessment for the EAS-modality, Scenario 2 a (ii) is the most appropriate Scenario (not 1b), as the dataset was considered sufficient for evaluation of endocrine activity for the EAS-modalities, and no consistent test substance-related effects were observed on EAS-mediated endocrine activity.	RMS: Comment acknowledged.  Please refer to comment 14.  The mistake was corrected.  Addressed.	Addressed.  See reply to comment 14.
20.	Page 509, Sensitive to, but not diagnostic of, EATS, last bullet point	APPL: The applicant considers that it would be useful to include that in [REDACTED] 2010 (Study ID 30) there were 6 fish per replicate (2 male and 4 female) so a mortality of 17%, represents one fish.	RMS: The data were included for the Study ID:30).  Addressed.	Addressed.  The addendum was clarified in accordance with the comment in column 2.
21.	Page 510, Overall assessment of EAS, 3 <sup>rd</sup> paragraph	APPL: The applicant agrees with the overall NTO EAS modality conclusion that ' <i>scenario 2a(ii) is applied and ED criteria are not met for EAS modalities for the active substance 2,4-D since there is no evidence of endocrine activity to support</i>	RMS: Comment acknowledged.  No further action is required.	Noted.

Potential for endocrine disruption				
No.	Column 1 Reference to Assessment Report (vol., point, page)	Column 2 Comments from Member States, EFSA, notifier or public	Column 3 Evaluation by (RMS) rapporteur and - if available - (Co-RMS) Co-rapporteur / response from the notifier	Column 4 Data requirement or Open point (if data point not addressed or fulfilled)
		<i>biological plausibility for an endocrine MoA.</i>		
22.	Page 510, Overall assessment of EAS, last paragraph	APPL: The applicant notes that the RMS comments ' <i>a level of uncertainty is raised from the findings in the offspring in the EOGRTs study</i> '. However in the RMS mammalian EAS modalities conclusion this uncertainty is not mentioned, and it is concluded that ' <i>Overall, no concern is identified for EAS-mediated adversity from studies with ID: 1, 2, 3, 4, 12, 13, 19 and 86.</i> ' The EOGRT study is ID: 19. The applicant suggest referring to the mammalian evaluation for any discussion on the EOGRT study observations.	RMS: The findings regarding the EOGRTs study were rephrased.  Addressed.	Addressed.  The addendum was clarified with regard to the findings in the EOGRT study with mammals.
23.	Page 510, Overall conclusion on the ED assessment	APPL: The applicant notes that the applicant overall conclusion on the ED assessment has not be included in the assessment.	RMS: Comment acknowledged. The overall assessment and conclusion by the RMS for non-target organisms was included in the addendum.  Addressed.	Addressed.  The conclusion on the ED assessment drawn by the RMS is included in the addendum which seems in line with the applicant's assessment based on the comments 18, 21 and 24.
24.	Page 510, Overall conclusion on the ED assessment, Assessment and conclusion by the RMS	APPL: The applicant agrees with the overall ED assessment conclusion that 'ED criteria are not met for EATS modalities on human health and the environment.'	RMS: Comment acknowledged.  No further action is required.	Noted.

Potential for endocrine disruption				
No.	Column 1 Reference to Assessment Report (vol., point, page)	Column 2 Comments from Member States, EFSA, notifier or public	Column 3 Evaluation by (RMS) rapporteur and - if available - (Co-RMS) Co-rapporteur / response from the notifier	Column 4 Data requirement or Open point (if data point not addressed or fulfilled)
25.	Various typographical errors	<p>APPL: The applicant suggests there are a small number of typographical errors.</p> <p>Page 487, Have EAS-mediated parameters been sufficiently investigated?: 3<sup>rd</sup> paragraph: font has changed from Tahoma to Calibri.</p> <p>Page 506, Table 22 WoE for EAS-mediated adversity and endocrine activity, 5<sup>th</sup> row: Start sentence with <b>I</b></p>	<p>RMS: Comment acknowledged. Typos were corrected.</p> <p>Addressed.</p>	<p>Addressed.</p> <p>Typos listed in column 2 have been corrected in the addendum.</p>

## Appendix B – Amended part of the LoEP

### Neurotoxicity (Annex IIA, point 5.7)

Additional studies (e.g. delayed neurotoxicity, developmental neurotoxicity ‡

No developmental neurotoxicity. NOAEL<sub>offspring</sub> 16.6 mg/kg bw per day [F1-extended one generation study]

### Other toxicological studies (Annex IIA, point 5.8)

Immunotoxicity

Developmental immunotoxicity in rat:  
No immunotoxicity. NOAEL<sub>offspring</sub> 16.6 mg/kg bw per day [F1-extended one generation study]

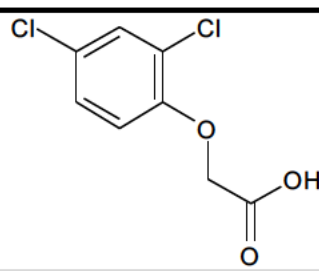
Endocrine disrupting properties

Criteria not met for the EAS and T modalities in a sufficiently investigated dataset.

### Endocrine disrupting properties (Annex Part A, points 8.1.5 and 8.2.3)

An Amphibian Metamorphosis Assay (AMA) and a Fish Short Term Reproduction Assay (FSTRA) were performed. Additionally, a number of studies retrieved through a systematic literature review were also available. Overall, the available evidence do not seem to show a pattern of endocrine activity of 2,4-D for non-mammalian species.

## Appendix C – Used compound codes

Code/trivial name <sup>(a)</sup>	IUPAC name/SMILES notation/InChiKey <sup>(b)</sup>	Structural formula <sup>(c)</sup>
<b>2,4-D</b>	(2,4-dichlorophenoxy)acetic acid <chem>Clc1cc(Cl)ccc1OCC(=O)O</chem> OVSKIKFHRZPJSS-UHFFFAOYSA-N	

(a): The compound/ metabolite name in bold is the name used in the conclusion.

(b): ACD/Name 2021.1.3 ACD/Labs 2021.1.3 (File Version N15E41, Build 123232, 07 Jul 2021)

(c): ACD/ChemSketch 2021.1.3 ACD/Labs 2021.1.3 (File Version C25H41, Build 123835, 28 Aug 2021)