Dimethomorph

Assessment of Potential Endocrine Disruption of the Active Substance

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[redacted]

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Summary

The active substance dimethomorph is under evaluation regarding its renewal of approval according to Regulation (EC) No 1107/2009. In this context, EFSA requested from the applicant an assessment according to the new criteria for endocrine disruptor (ED) laid down in Commission Regulation (EU) No 2018/60 and following the new EFSA/ECHA Guidance Document for the identification of ED.

For ED assessment of dimethomorph in humans and mammals, slight *in vivo* effects were observed in the pups of the extended 1-generation study:

- Changes in anogenital distance/index of males at the high dose
- Delayed sexual maturation of males at the high dose
- Decreased seminal vesicle weight at the high dose

These effects are observed in the presence of systemic toxicity (general growth retardation).

These parameters could be associated with endocrine activity: anti-androgenicity, also observed in *in vitro* tests at high doses.

However, other effects characteristics of anti-androgenic substances were <u>not</u> observed, i.e. dimethomorph had <u>no</u> effect on the following parameters:

- sperm abnormalities,
- hormonal changes,
- malformations of the male sexual tract,
- nipple retention,
- histopathological alterations in endocrine organs

According to Commission Regulation 2018/605 (and the WHO definition) an adverse effect is defined as "*a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population* ***that result in*** *an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences.*". The slight observed effects cannot be considered as adverse per see.

To conclude on the adversity, functional impairment of the reproduction of the treated pups should be demonstrated. In the 2-generation study, no effects were observed, but in this study the highest dose was about 70 mg/kg bw/d and thus lower than the highest dose (about 140 mg/kg bw/d) in the extended 1-generation.

To clarify this uncertainty concerning adversity**, it is proposed to perform a new extended 1-generation study with additional cohorts** (especially a second-generation cohort as mentioned in the ED guidance).

Currently, following the ED guidance document, **it is not possible to have a firm conclusion on the ED** properties of dimethomorph for **humans and (wild) mammals**.

For the ED assessment of dimethomorph in non-target organisms other than mammals, a fish short-term reproduction assay (FSTRA) was conducted to specifically address the anti-androgenic activity of dimethomorph indicated in the mammalian screening *in vitro* assays. The FSTRA demonstrated that dimethomorph has no endocrine activity, including anti-androgenicity, in fish. Accordingly, no biologically plausible link could be established between the activity of dimethomorph observed in the mammalian screening *in vitro* assays and an adverse effect in aquatic vertebrates. In conclusion, **dimethomorph does not meet the ED criteria in aquatic vertebrates** in line with Commission Regulation (EU) No 2018/60 amending the Plant Protection Regulation No. 1107/2009 because dimethomorph does not show adverse effects, which could be consequence of an endocrine

mode of action. In line with the ECHA/EFSA ED GD (2018) the EAS modalities for non-target organisms other than mammals have been sufficiently investigated with the FSTRA. The T-modality is not of concern in case of dimethomorph as shown by the existing mammalian dataset. Based on the existing dataset it appears highly unlikely that dimethomorph fulfils ED criteria in birds. In conclusion, there is no need to generate further data to conclude on the ED potential of dimethomorph in non-target organisms other than mammals and **no further studies with non-target organisms other than mammals are proposed.**

1. Background

The objective of this report is to assess the potential endocrine disruption (ED) properties of dimethomorph in the context of Regulations (EC) No 1107/2009 following the ED criteria laid down in Commission Regulation (EU) No 2018/605. The document is prepared to address a request of EFSA for an updated ED assessment according to the new EFSA/ECHA Guidance[1] for the evaluation of the active substance dimethomorph in the course of the AIR process.

Following the BASF's request, EFSA has kindly granted an adequate time extension till December 14th, 2018, in order to be able to:

- Update the already performed literature search: Doc ID **2018/1208894** for Toxicology and included in the Toxicology folder, Doc ID **2018/1193073** and **2018/1227435** for Ecotoxicology and included in the Ecotoxicology folder.
- Perform for the toxicology area a specific search on Toxcast and Tox21 database, the results are reported in the document Doc ID **2018/1196406**.
- Fulfill Appendix E, as described in the Guidance documents. There are two Appendix E, one for Toxicology and Ecotoxicology Data, Doc ID **2018/1202685**, included in the General folder, and one for Ecotoxicology Data only, Doc ID **2018/1227434** because of some technical issues, and located in the Ecotoxicology folder.

All the references reported in the Assessment are located in the folder 4. References

This document also includes a proposal of generating further data to complete the ED assessment of dimethomorph according to the requirements of the guidance by ECHA/EFSA.

1.1. Definitions and criteria for endocrine disruptors

According to Commission Regulation 2018/605 amending the Plant Protection Regulation No. 1107/2009 a substance shall be considered as having endocrine disrupting properties if it meets the following criteria:

1) It shows an adverse effect in an intact organism or its progeny, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population **that results in** an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences.

2) It has an endocrine mode of action (MoA), *i.e.* it alters the function of the endocrine system

3) The adverse effect on humans or on populations of non-target organisms is a consequence of the endocrine MoA.

[1] 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.

In order to consider a substance as an endocrine disruptor, it needs to meet all three criteria for humans or non-target organisms. In most cases the key question of the ED assessment concerns the third criterion, i.e. if there is a biologically plausible link between endocrine activity and observed adverse effect(s).

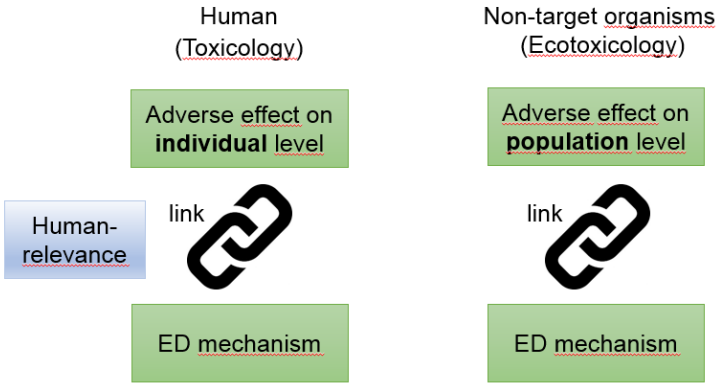


Fig. 1: Graphical Illustration of the ED criteria laid down in Commission Regulation 2018/605.

2. Endocrine disruption assessment

2.1. Gather information.

According to the EFSA/ECHA ED GD (2018) all available 'scientific data' covering *in vivo* studies predictive for adverse effects as well *in vitro* studies informing about endocrine activity and mechanism should be taken into consideration.

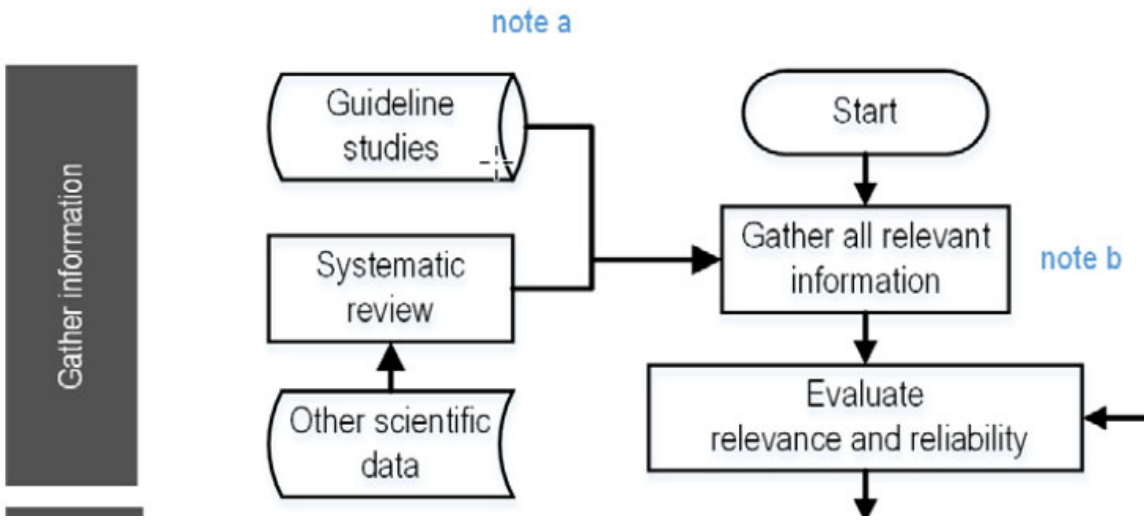


Fig. 2: Excerpt of the flowchart shown on page 13 of the ECHA/EFSA Guidance Document

The main source for the ED assessment of dimethomorph are the standard regulatory studies conducted to fulfil the data requirements of Commission Regulation 283/2013 for the approval of the active substance under Regulation 1107/2009 (see dimethomorph dossier 2016, Document M of Annex II (Doc CA) and respective study reports).

In addition, the available ToxCast/Tox21 data on dimethomorph as they relate to endocrine activity was collected and summarized (see DocID 2018/1196406). Dimethomorph has not been part of the US EPA

endocrine screening program (EDSP) and accordingly there is no data on dimethomorph in the US EPA EDSP21 Dashboard for E, A and T modalities.

A systematic review of literature for dimethomorph was already conducted and submitted with the EU dossier in January 2016 in line with the guidance document of EFSA on submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. In the context of this ED assessment a further literature search was performed by the BASF Scientific Information Center. Different search profiles were used to identify literature relevant for dimethomorph with keywords of the categories ecotoxicology, toxicology, mode of action, QSAR and endocrine. The databases and the keywords used in the different search profiles are summarized in the literature search report (BASF Scientific Information Center, DocID 2018/1208894) submitted with this assessment.

The updated literature search resulted in no relevant data for the ED assessment of dimethomorph for human health and non-target organisms (for a detailed description of the criteria used in the assessment of relevance and reliability please see BASF DocID 2018/1193073). The detailed assessment on relevance and reliability of the published literature on ED in wild mammals and non-target organisms other than mammals is given in the attached excel file (DocID 2018/1227435).

All studies that are relevant for the ED assessment of dimethomorph in humans and mammals are listed in Table 1.

Table 1: Studies for ED assessment of dimethomorph in humans and mammals.

Study ID*	Study type	Species	Guideline
1	Repeated Dose 28-Day Oral Toxicity in rodents	Rat (Sprague-Dawley)	OECD 407
2	Repeated Dose 28-Day Oral Toxicity in rodents	Rat (Sprague-Dawley)	OECD 407
3	Repeated Dose 28-Day Oral Toxicity in rodents	Rat (Fischer 344)	OECD407
4	Repeated Dose 28-Day Oral Toxicity in rodents	Rat (Fischer 344)	OECD 407
5	Repeated Dose 90-Day Oral Toxicity in rodents	Rat (Sprague-Dawley)	OECD 408
6	Sub-acute repeat dose toxicity Oral 6-week in mouse	Mouse (CD-1)	Range-finding
7	Sub-acute repeat dose toxicity Oral 90-day study in dogs	Beagle	OECD 409
8	Sub-acute repeat dose toxicity Oral 1-year study in dogs	Beagle	OECD 409
9	Sub-acute repeat dose toxicity Dermal 28-day study in rats	Rat (Wistar)	OECD 410
10	Chronic toxicity Oral in rats	Rat (Sprague-Dawley)	OECD 452
11	Carcinogenicity Oral in rats	Rat (Sprague-Dawley)	OECD 451
12	Carcinogenicity Oral in mice	Mouse (CD-1)	OECD 451
13	Two-Generation Reproduction Toxicity	Rat (Sprague-Dawley)	OECD 416
14	Extended One-Generation Reproductive Toxicity	Rat (Sprague-Dawley)	OECD 443
15	Prenatal developmental toxicity study	Rat (Sprague-Dawley)	OECD 414
16	Prenatal developmental toxicity study	Rat (Sprague-Dawley)	Range-finding
17	Prenatal developmental toxicity study	Rabbit (NZ white)	OECD 414
18	Prenatal developmental toxicity study	Rabbit (NZ white)	Range-finding
19	90-day neurotoxicity in rodents.	Rat (Wistar)	OECD 424
20	28-day immunotoxicity in rodents	Rat (Wistar)	EPA 870,7800
21	AR Binding Assay	Yeast cells (Saccharomyces cerevisiae)	

22	ER Binding Assay	Yeast cells (Saccharomyces cerevisiae)	
23	Stably Transfected Human ERα Transcriptional Activation Assay (ER STTA)	Human breast cancer cells (MCF-7)	
24	AR Binding Assay	Yeast cells	
24	Stably Transfected Human AR Transactivation Assay (AR STTA)	MDA-kb2	
25	Stably Transfected Human AR Transactivation Assay (AR STTA)	MDA-kb2	
26	AR Binding Assay	Yeast cells	
27-84	ToxCast *in vivo* assays		

*Study ID in Appendix E

Table 2 gives the studies on non-target organisms other than mammals that are relevant for the ED assessment of dimethomorph and were reviewed in the dimethomorph data package. Please note that the ED assessment for wild mammals is based on the data package for mammals (see Table 1).

Table 2: Studies for ED assessment of dimethomorph in non-target organisms other than mammals.

Study ID*	Study type	Species	Guideline
85	fish short-term reproduction assay (FSTRA)	fathead minnow (*Pimephales promelas*)	OECD TG 229
86	fish early life-stage tests (ELS)	rainbow trout (*Oncorhynchus mykiss*)	OECD TG 210
87	fish early life-stage tests (ELS)	fathead minnow (*Pimephales promelas*)	OECD TG 210
88	fish early life-stage tests (ELS)	sheepshead minnow (*Cyprinodon variegatus*)	OECD TG 210
89	prolonged (21-d) toxicity	rainbow trout (*Oncorhynchus mykiss*)	OECD TG 204
90	avian reproduction tests	mallard (*Anas platyrhynchos*)	OECD TG 206
91	avian reproduction tests	bobwhite quail (*Colinus virginianus*)	OECD TG 206

*Study ID in Appendix E

All relevant studies on mammals, birds and fish were gathered and reported in the format of an Excel File as attached to the Guidance document as Appendix E (see EFSA/ECHA ED GD 2018). There are two Excel Files: (1) Appendix E with all relevant data (DocID 2018/1202685) and (2) Appendix E with ecotoxicological studies only[2] (EFSA ED GD_Appendix-E1_DMM_ecotox data_xlsx, DocID 2018/1227434). In the overview table ('data summary') all studies are listed including all endpoints assigned to the following effect categories:

- *In vitro* mechanistic
- *In vivo* mechanistic
- 'EATS'-mediated
- 'Sensitive to, but not diagnostic of EATS'
- General adversity

[2] In the Appendix E with all relevant data, not all parameters from the fish studies that were entered in the "data" sheet occur in the "data summary" (i.e. vitellogenin in males and females is missing). In Appendix E with ecotoxicological studies only no parameter is missing in the data summary. For this reason, the second Excel file "Appendix E "EFSA ED GD_Appendix-E1_DMM_ecotox data" is provided that no relevant information gets lost.

2.2. Assess the evidence.

The relevant and reliable information was assembled into lines of evidence, integrating information for both adversity and endocrine activity according to the guidance. As a result, Table 3 and Table 4 for the mammalian data package, Table 5 for aquatic vertebrates, and Table 6 for birds were obtained.

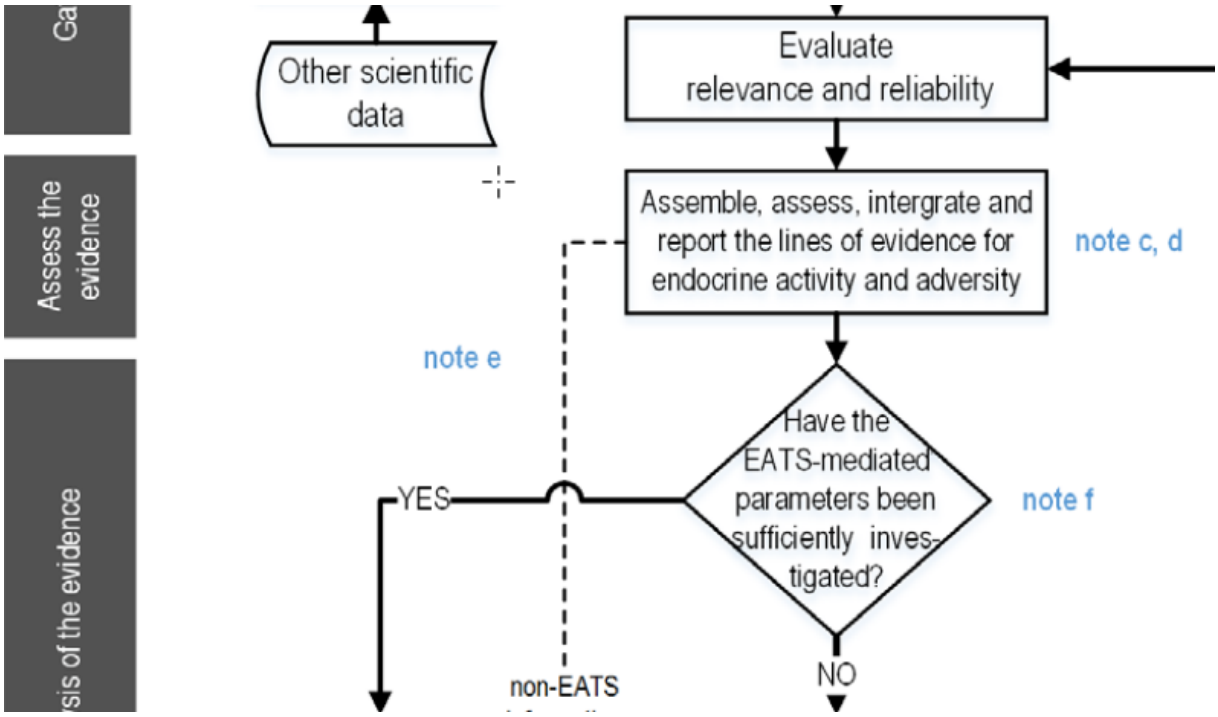


Fig. 3: Excerpt of the flowchart shown on page 13 of the ECHA/EFSA Guidance Document

Table 3: Lines of evidence for estrogen/androgen and steroidogenesis modality after dimethomorph exposure in mammals

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
Integrated lines of evidence for endocrine activity									
In silico predictions	No data								
In vitro mechanistic	ToxCast –AR	human cell lines (kidney, liver and ovary)				No effect	No effect on the androgen pathway	Some evidence for anti-androgenic activity *in vitro;* Weak/no evidence for S-related activity *in vitro*; No evidence for E-related activity	E, A, S
	ToxCast - ER	human cell lines (kidney and liver)				No effect	No effect on the estrogen pathway		
	ToxCast - S	human cell lines				Upregulation of estrone and estradiol	Effects on S pathway discussed in the respective §		
	ToxCast - aromatase	human cell lines				No effect	No effect		
In vitro mechanistic	YES assay					Negative	No effect		
	YAS assay					Positive at high doses	Slight anti-androgenic effects at high dose *in vitro*		
	YAS assay					Positive at high doses			
	YAS assay					Positive at high doses			
	AR human					Positive at high doses			
Integrated lines of evidence for endocrine adversity									
EATS mediated parameter	Age at preputial separation	Rat	1-generation	Oral	800 ppm	Increase at 800 and 1600 ppm. Effects seen at 800 ppm are clearly attributed to growth retardation.	In the high dose, slight effect on age of preputial separation could be attributed to an anti-androgenic activity.	Slight anti-androgenic activity was observed at the high dose in the extended 1-generation study	
	Age at vaginal opening	Rat	1-generation	Oral	1600 ppm	No effects at the highest dose	No effect	No effect	
	Anogenital distance	Rat	1-generation	Oral	800 ppm	Decrease in male anogenital distance index at 800 and 1600 ppm. 800 ppm effect clearly related to lower body weight	In the high dose, slight effect on AGD/AGI could be attributed to an anti-androgenic activity.	Slight anti-androgenic activity was observed at the high dose in the extended 1-generation study	
	Cervix (histopathology)	Rat	2-generation	Oral	1000 ppm	No effects at the highest dose	No effect	No effect	
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Coagulating gland (histopathology)	Rat	2-generation	Oral	1000 ppm	No effects at the highest dose	No effect	No effect	
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
	Epididymis weight	Dog	90 days	Oral	1350 ppm	No effects at the highest dose	No effect	No significant effect on epididymis in all species.	
		Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose	No effect		
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose	No effect		
		Rat	1-generation	Oral	1600	Decrease absolute epididymis weight only at the high dose (not relative)	Effect can be link to general toxicity (growth retardation) especially because no effect was observed in the relative weight. Moreover, no histopathological effects were observed at this dose level		
	Epididymis (histopathology)	Dog	90 days	Oral	1350 ppm	No effects at the highest dose	No effect		
		Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose			
		Mouse	104 weeks	Oral	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Estrus cyclicity	Rat	90 days	Oral	1000 ppm	No effects at the highest dose	No effect	No effect on oestrus cyclicity	
		Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Genital abnormalities	Rat	1-generation	Oral	1600 ppm	No effects at the highest dose	No effect	No effect on natal abnormalities	
	Male mammary gland histopathology	Rat	1-generation	Oral	1600 ppm	No effects at the highest dose	No effect	No effect on male or female mammary glands	
	Mammary gland histopathology	Mouse	104 weeks	Oral	1000 mg/kg bw/d	No effects at the highest dose	No effect		
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Nipple development	Rat	1-generation	Oral	1600 ppm	No effects at the highest dose	No effect	No effect on nipple development	
	Ovary weight	Dog	90 days	Oral	1350 ppm	No effects at the highest dose	No effect	No effects on ovaries	
		Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose			
		Mouse	6 weeks	Oral	4000 ppm	No effects at the highest dose			
		Rat	28 days	Oral	5000 ppm	No effects at the highest dose			
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Ovary (histopathology)	Dog	90 days	Oral	1350 ppm	No effects at the highest dose	No effect		
		Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose			
		Mouse	104 weeks	Oral	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Oviduct (histopathology)	Rat	1-generation	Oral	1600 ppm	No effects at the highest dose	No effect	No effect on oviduct	
	Prostate weight	Dog	90 days	Oral	1350 ppm	Decrease at the highest dose	Decreased prostate weight (absolute and relative) was observed at the high dose	Prostate effect was observed in one rat study and in both dog studies. In rat a slight decrease was observed in the extended 1-	
		Dog	52 weeks	Oral	1350 ppm	Decrease at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose	No effect		

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
		Rat	1-generation	Oral	300 ppm	Decrease absolute prostate weight at all doses, decrease relative prostate weight at 800 and higher. Only in adults F1	Effect on prostate weight can be related to treatment concurrently with general systemic effects on body weight. No histopathology was associated with. Changes were within the HCD.	generation, within the HCD and without histopathology. This effect is therefore considered as not related to ED properties. In dogs a slight decrease of the prostate weight was observed at the high dose. Histopathology (interstitial fibrosis was observed at the high dose of the 90-day study and in all doses of the 1-year study confirming that this effect is common is ageing dogs. A slight increase of severity was observed in the 1-year dog study but this is attributed to the histological technique and not related to endocrine activity.	
	Prostate (histopathology)	Dog	90 days	Oral	150 ppm	Changes at the highest dose	Increase of interstitial fibrosis was observed in the high dose.		
		Dog	52 weeks	Oral	1350 ppm	Changes at all doses	Increase of interstitial fibrosis was observed in all doses including control. This slight increase in severity apparently observed can be attributed the the histopathology technique.		
		Mouse	104 weeks	Oral	1000 mg/kg bw/d	No effects at the highest dose	No effect		
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
	Seminal vesicle weight	Rat	1-generation	Oral	800 ppm	Decrease of absolute weight (not relative)	Decrease of absolute seminal weight (not relative) is attributed to the lower body weight of the pups at these dose level	A relative decrease of the absolute weight was observed in pups of the extended 1-generation study together with effects on body weight. As no absolute change was observed and as no histopathological findings were observed in all other studies, this effect is due to systemic toxicity without a link to endocrine activity.	
	Seminal vesicles (histopathology)	Rat	1-generation	Oral	1600 ppm	No effects at the highest dose	No effect		
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
	Sperm morphology	Rat	1-generation	Oral	1600 ppm	No effects at the highest dose	No effect	No effect on sperm morphology	
	Sperm motility	Rat	1-generation	Oral	1600 ppm	No effects at the highest dose	No effect	No effect on sperm motility	
	Sperm number	Rat	1-generation	Oral	1600 ppm	No effects at the highest dose	No effect	No effect on sperm number	
	Testis weight	Dog	90 days	Oral	1350 ppm	No effects at the highest dose	No effect	Testes effects were observed in one dog study and 3 rat studies. Weight changes were observed (only relative) that can be link to general toxicity and not related to endocrine activity. For testes adenomas in long term rat studies,	
		Dog	52 weeks	Oral	450	Increase testis weight	Relative increase at the mid- and-high dose. No changes in absolute weight.		
		Rat	28 days	Oral	5000 ppm	No effects at the highest dose	No effect		
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose	No effect		
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose	No effect		
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose	No effect		
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose	No effect		

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose	No effect	this is a common finding in this strain of rats and is not considered to be substance related. No endocrine activity can be link to this effect.	
		Rat	1-generation	Oral	800 ppm	Increase relative weight only (not absolute)	Relative increase at the mid- and-high dose. No changes in absolute weight. Concurrently with lower body weight.		
	Testis (histopathology)	Dog	90 days	Oral	1350 ppm	No effects at the highest dose	No effect		
		Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose	No effect		
		Mouse	104 weeks	Oral	1000 mg/kg bw/d	No effects at the highest dose	No effect		
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose	No effect		
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose	No effect		
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose	No effect		
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose	No effect		
		Rat	2 years	Oral	2000 ppm	Adenomas in the high dose	Increase of testis adenomas in the high dose. Considered not substance related because of the higher lifespan and not statistically significant (see assessment in §2.3.1)		
		Rat	2 years	Oral	2000 ppm	Adenomas in the high dose			
		Rat	2-generation	Oral	1000 ppm	No effects at the highest dose	No effect		
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose	No effect		
	Uterus weight	Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose	No effect	Slight decrease of weight in only one study at a very high dose associated with systemic toxicity and without	
		Rat	28 days	Oral	5000 ppm	decrease	Decrease at the highest dose		
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose	No effect		
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose	No effect		

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose	No effect	histopathology correlate; it is considered to be not endocrine mediated.	
	Uterus (histopathology)	Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose			
		Mouse	104 weeks	Oral	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Vagina (histopathology)	Mouse	104 weeks	Oral	1000 mg/kg bw/d	No effects at the highest dose	No effect	No histopathological effects in vagina	
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
Sensitive to, but not diagnostic of, EATS	Adrenal weight	Dog	90 days	Oral	1350 ppm	No effects at the highest dose	An increase of adrenal weight was observed in only one rat study. This finding was not observed in all other studies. This is considered as incidental as no histopathology was observed.	No effects on adrenals	
		Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose			
		Mouse	6 weeks	Oral	4000 ppm	No effects at the highest dose			
		Rat	28 days	Oral	5000 ppm	No effects at the highest dose			
		Rat	28 days	Oral	100 mg/kg bw/d	Increase			
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Adrenal histopathology	Dog	90 days	Oral	1350 ppm	No effects at the highest dose			
		Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose			
		Mouse	104 weeks	Oral	1000 mg/kg bw/d	No effects at the highest dose			

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Fertility	Rat	2-generation	Oral	1000 ppm	No effects at the highest dose	No effects on fertility	No effects on fertility	
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Dystocia	Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Gestation length	Rat	2-generation	Oral	1000 ppm	No effects at the highest dose	No effect	The effects observed on the gestation length in the extended 1-generation study are considered as a chance finding. Duration of gestation of individual dams varies between 21 and 23 days, concurrent controls are in the upper range of HCD and the statistical significance of the effect in the high dose is unclear. This effect cannot be linked to any endocrine mode of action.	
		Rat	1-generation	Oral	1600 ppm	Very slight decrease at the highest dose	Slight decrease of gestation length at the high dose.		
	Birth index	Rat	2-generation	Oral	1000 ppm	No effects at the highest dose	No effects on reproduction. The few observations noted	Concerning reproduction functions, the few observations	
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Foetal development	Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
	Lactation index	Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose	can be attributed to maternal toxicity (reduced weight gain, weight loss)	noted can be attributed to maternal toxicity and not to an endocrine activity.	
	Litter size	Rabbit	Developmental study	Oral	650 mg/kg bw/d	No effects at the highest dose			
		Rabbit	Developmental study	Oral	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
		Rat	Developmental study	Oral	160 mg/kg bw/d	No effects at the highest dose			
		Rat	Developmental study	Oral	300 mg/kg bw/d	No effects at the highest dose			
	Litter viability	Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Number of implantation, corpora lutea	Rabbit	Developmental study	Oral	650 mg/kg bw/d	No effects at the highest dose			
		Rabbit	Developmental study	Oral	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
		Rat	Developmental study	Oral	160 mg/kg bw/d	No effects at the highest dose			
		Rat	Developmental study	Oral	300 mg/kg bw/d	No effects at the highest dose			
	Number of live births	Rabbit	Developmental study	Oral	650 mg/kg bw/d	Decrease at the highest dose			
		Rabbit	Developmental study	Oral	1000 mg/kg bw/d	Decrease at the highest dose			
		Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Number of ovarian follicles	Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Pituitary weight	Dog	90 days	Oral	1350 ppm	No effects at the highest dose			
		Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose			
		Mouse	6 weeks	Oral	4000 ppm	No effects at the highest dose			
		Rat	28 days	Oral	1000 ppm	Decrease			
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
	Pituitary histopathology	Dog	90 days	Oral	1350 ppm	No effects at the highest dose			
		Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose			
		Mouse	104 weeks	Oral	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	Decrease			
		Rat	2 years	Oral	2000 ppm	Decrease			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Post implantation loss	Rabbit	Developmental study	Oral	650 mg/kg bw/d	No effects at the highest dose			
		Rabbit	Developmental study	Oral	1000 mg/kg bw/d	Increase at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
		Rat	Developmental study	Oral	160 mg/kg bw/d	Increase at the highest dose			
		Rat	Developmental study	Oral	300 mg/kg bw/d	No effects at the highest dose			
	Pre implantation loss	Rabbit	Developmental study	Oral	650 mg/kg bw/d	Increase at the highest dose			
		Rabbit	Developmental study	Oral	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
		Rat	Developmental study	Oral	160 mg/kg bw/d	No effects at the highest dose			
		Rat	Developmental study	Oral	300 mg/kg bw/d	No effects at the highest dose			
	Pup development	Rabbit	Developmental study	Oral	650 mg/kg bw/d	No effects at the highest dose			
		Rabbit	Developmental study	Oral	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
		Rat	Developmental study	Oral	160 mg/kg bw/d	No effects at the highest dose			
		Rat	Developmental study	Oral	300 mg/kg bw/d	No effects at the highest dose			

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
	Pup survival index	Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Sex ratio	Rabbit	Developmental study	Oral	650 mg/kg bw/d	No effects at the highest dose			
		Rabbit	Developmental study	Oral	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
		Rat	Developmental study	Oral	160 mg/kg bw/d	No effects at the highest dose			
		Rat	Developmental study	Oral	300 mg/kg bw/d	No effects at the highest dose			
	Time to mating	Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
Evidence of general toxicity	Mortality	Dog	90 days	Oral	1350 ppm	No effects at the highest dose	Mortality was higher in 2 studies (one in rabbit at the dose of 1000 mg/kg bw/d and one in rat at the dose of 5000 ppm). Mortality was decreased in the long-term studies in rats.	Systemic toxicity was observed at very high dose in rats and rabbits.	n.a.
		Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose			
		Mouse	6 weeks	Oral	4000 ppm	No effects at the highest dose			
		Mouse	104 weeks	Oral	1000 ppm	No effects at the highest dose			
		Rabbit	Developmental study	Oral	650 mg/kg bw/d	No effects at the highest dose			
		Rabbit	Developmental study	Oral	1000 mg/kg bw/d	Increase at the highest dose			
		Rat	28 days	Oral	5000 ppm	Increase at the highest dose			
		Rat	28 days	Oral	4000 ppm	No effects at the highest dose			
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	Decrease at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
		Rat	Developmental study	Oral	160 mg/kg bw/d	No effects at the highest dose			
		Rat	Developmental study	Oral	300 mg/kg bw/d	No effects at the highest dose			

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
		Rat	90 days neurotoxicity	Oral	2400 ppm	No effects at the highest dose			
		Rat	28 days immunotoxicity	Oral	2400 ppm	No effects at the highest dose			
	Systemic toxicity	Dog	90 days	Oral	1350 ppm	Increase at the highest dose	Clinical signs of systemic toxicity were observed in each species. From 1350 ppm in dogs, 1000 mg/kg bw/d in rabbits and 2000 ppm in rats.	Clinical signs of toxicity were observed in all species at relatively high doses.	
		Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose			
		Mouse	6 weeks	Oral	4000 ppm	No effects at the highest dose			
		Mouse	104 weeks	Oral	1000 ppm	No effects at the highest dose			
		Rabbit	Developmental study	Oral	650 mg/kg bw/d	No effects at the highest dose			
		Rabbit	Developmental study	Oral	1000 mg/kg bw/d	Increase at the highest dose			
		Rat	28 days	Oral	5000 ppm	Increase at the highest dose			
		Rat	28 days	Oral	4000 ppm	Increase at the highest dose			
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	Increase at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	2-generation	Oral	1000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Food consumption	Mouse	6 weeks	Oral	4000 ppm	No effects at the highest dose	Decrease in food consumption was observed in many feeding studies and also in gavage studies. For gavage studies, this effect appears from 650 mg/kg bw/d in rabbits and 160 mg/kg bw/d in rats. For feeding studies, decrease was seen at all doses in the extended 1-generation study, and at higher	Decrease in food consumption associated with decrease ion body weight was observed in all species. And was more pronounced with the study duration.	
		Rabbit	Developmental study	Oral	650 mg/kg bw/d	Decrease at the highest dose			
		Rabbit	Developmental study	Oral	1000 mg/kg bw/d	Decrease at the highest dose			
		Rat	28 days	Oral	5000 ppm	Decrease at the highest dose			
		Rat	28 days	Oral	2000 ppm	Decrease at 2000 and 4000 ppm			
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			
		Rat	2-generation	Oral	1000 ppm	Decrease at the highest dose			
		Rat	1-generation	Oral	300 ppm	Decrease at the all doses			
		Rat	Developmental study	Oral	160 mg/kg bw/d	Decrease at the highest dose			
		Rat	Developmental study	Oral	300 mg/kg bw/d	No effects at the highest dose			
		Rat	90 days neurotoxicity	Oral	2400 ppm	Decrease at the highest dose			

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
		Rat	28 days immunotoxicity	Oral	2400 ppm	No effects at the highest dose	doses in other studies depending on the time of treatment.		
	Body weight	Dog	90 days	Oral	1350 ppm	No effects at the highest dose	Effects on body weight were observed in many studies, mainly mid- and long - term studies in all species.		
		Dog	52 weeks	Oral	450 ppm	Decrease at 450 and 1350			
		Mouse	6 weeks	Oral	4000 ppm	No effects at the highest dose			
		Mouse	104 weeks	Oral	1000 ppm	Decrease at the highest dose			
		Rabbit	Developmental study	Oral	650 mg/kg bw/d	Decrease at the highest dose			
		Rabbit	Developmental study	Oral	300mg/kg bw/d	Decrease at all doses			
		Rat	28 days	Oral	5000 ppm	Decrease at the highest dose			
		Rat	28 days	Oral	2000 ppm	Decrease at all doses			
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	Decrease at the highest dose			
		Rat	2 years	Oral	750 ppm	Decrease at 750 and 2000 ppm			
		Rat	2-generation	Oral	1000 ppm	Decrease at the highest dose			
		Rat	1-generation	Oral	1600 ppm	Decrease at 800 and 1600 (pups)			
		Rat	Developmental study	Oral	160 mg/kg bw/d	Decrease at the highest dose			
		Rat	Developmental study	Oral	300 mg/kg bw/d	No effects at the highest dose			
		Rat	90 days neurotoxicity	Oral	2400 ppm	Decrease at the highest dose			
		Rat	28 days immunotoxicity	Oral	2400 ppm	Decrease at the highest dose			
	Brain weight	Dog	90 days	Oral	1350 ppm	No effects at the highest dose	No effect	No adverse effect on brain.	
		Dog	52 weeks	Oral	450 ppm	No effects at the highest dose			
		Mouse	6 weeks	Oral	4000 ppm	No effects at the highest dose			
		Rat	28 days	Oral	5000 ppm	No effects at the highest dose			
		Rat	28 days	Oral	4000 ppm	No effects at the highest dose			
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	800 ppm	Increase relative weight at 800 and 1600 (pups)	Increase relative brain weight can be linked to the general lower body weight due to systemic toxicity. This is not considered as an adverse effect.		
		Rat	90 days neurotoxicity	Oral	2400 ppm	No effects at the highest dose	No effect		
	Brain histopathology	Rat	28 days	Oral	4000 ppm	No effects at the highest dose	No effect		
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
		Rat	90 days neurotoxicity	Oral	2400 ppm	No effects at the highest dose			
	Haematological parameters	Dog	90 days	Oral	1350 ppm	No effects at the highest dose	Some slight effects were observed in blood parameters in some rat studies.	Overall there is some slight evidence that dimethomorph could have effect on haematological parameters at high doses in the rat. It cannot be considered as the main target organ.	
		Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose			
		Mouse	6 weeks	Oral	4000 ppm	No effects at the highest dose			
		Mouse	104 weeks	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Oral	5000 ppm	Increase platelet count at the highest dose			
		Rat	28 days	Oral	750 mg/kg bw/d	Decrease total blood at the highest dose			
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	90 days	Oral	1000 ppm	Decrease WBC at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	Decrease red blood cells at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
	Kidney weight	Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose	In 2 long term studies kidney weight were affected, this effect is observed	There is slight effect on kidney weight in long-term rat studies but without any	
		Rat	28 days	Oral	5000 ppm	No effects at the highest dose			
		Rat	28 days	Oral	4000 ppm	No effects at the highest dose			
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose	concurrently with body weight effects.	histopathological correlate. Kidney is not considered as a target organ.	
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	Increase at the highest dose			
		Rat	1-generation	Oral	1600 ppm	Increase (relative) at the highest dose			
	Kidney histopathology	Rat	28 days	Oral	4000 ppm	No effects at the highest dose	No effect		
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose	Liver weight is increased in all mid- long-term studies in rats.	Changes in liver weight associated with histopathological findings are observed in rats and dogs. This is the main target organ for dimethomorph toxicity	
	Liver weight	Dog	90 days	Oral	1350 ppm	No effects at the highest dose			
		Dog	52 weeks	Oral	1350 ppm	Increase at the highest dose			
		Mouse	6 weeks	Oral	4000 ppm	Increase at the highest dose			
		Rat	28 days	Oral	5000 ppm	Increase at the highest dose			
		Rat	28 days	Oral	2000 ppm	Increase at 2000 ppm and higher			
		Rat	28 days	Oral	1000 mg/kg bw/d	Increase at 100 mg/kg bw/d and higher			
		Rat	28 days	Oral	100 mg/kg bw/d	Increase at 100 mg/kg bw/d and higher			
		Rat	90 days	Oral	1000 ppm	Increase at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	Increase (absolute) at the highest dose			
	Liver histopathology	Dog	90 days	Oral	1350 ppm	No effects at the highest dose	Histopathological liver effects were observed in almost all long-term studies in rats and dogs.		
		Dog	52 weeks	Oral	1350 ppm	Increase at the highest dose (lipids)			
		Mouse	6 weeks	Oral	4000 ppm	No effects at the highest dose			
		Mouse	104 weeks	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Oral	2000 ppm	Increase at 2000 ppm and higher (hypertrophy)			
		Rat	28 days	Oral	1000 mg/kg bw/d	Increase at 100 mg/kg bw/d and higher			
		Rat	28 days	Oral	100 mg/kg bw/d	Increase at 100 mg/kg bw/d and higher (hepatocellular cytoplasmic lipid vacuolation)			
		Rat	90 days	Oral	1000 ppm	No effect at the highest dose			

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose ppm	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modalities
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	Increase at the highest dose			
		Rat	2 years	Oral	2000 ppm	Changes at the highest dose			
		Rat	1-generation	Oral	1600 ppm	Changes at the highest dose			
	Lung weight	Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose	No effect	No effects on lungs	
		Rat	28 days	Oral	4000 ppm	No effects at the highest dose			
	Lung histopathology	Rat	28 days	Oral	4000 ppm	No effects at the highest dose	No effect		
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
	Spleen weight	Dog	52 weeks	Oral	1350 ppm	No effects at the highest dose	Spleen weight was affected in 2 rat studies at high doses	Spleen weight effects were observed in some rat studies without histological correlates. These effects are not considered adverse and kidney is not a target organ.	
		Rat	28 days	Oral	5000 ppm	No effects at the highest dose			
		Rat	28 days	Oral	2000 ppm	No effects at the highest dose			
		Rat	28 days	Oral	750 mg/kg bw/d	Decease at the highest dose			
		Rat	28 days	Oral	750 mg/kg bw/d	No effects at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effect at highest dose			
		Rat	1-generation	Oral	1600 ppm	Decrease (absolute) at the highest dose			
	Spleen histopathology	Rat	28 days	Oral	2000 ppm	No effect at highest dose	No effect		
		Rat	28 days	Oral	750 mg/kg bw/d	No effect at highest dose			
		Rat	28 days	Oral	750 mg/kg bw/d	No effect at highest dose			
		Rat	1-generation	Oral	1600 ppm	No effect at highest dose			
	Biochemical changes	Dog	90 days	Oral	1350 ppm	Increase AP at the highest dose	Biochemical changes associated with liver toxicity were observed when measured in rat studies.	Effects on liver are also confirmed by biochemical changes. Confirming that the liver is the main target organ.	
		Dog	52 weeks	Oral	1350 ppm	Increase AP at the highest dose			
		Rat	28 days	Oral	1000 ppm	Decrease total plasma at 1000 ppm and higher			

Table 4: Lines of evidence for Thyroid modalities after dimethomorph exposure in mammals

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposure	Effect dose	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
Integrated lines of evidence for endocrine activity									
In vitro mechanistic	ToxCast –TR	human (liver) and rat (pituitary) cell line				No effects	No effect	No evidence for activity	T
	T3 and T4 level	Rat	1-generation	Oral	1600 ppm	No effects at the highest dose	No effect	No effects on thyroid	
	Thyroid stimulating hormone	Rat	1-generation	Oral	1600 ppm	No effects at the highest dose	No effect		
Integrated lines of evidence for endocrine adversity									
EATS mediated parameter	Thyroid weight	Dog	90 days	Oral	1350 ppm	No effects at the highest dose	No effect except in the 1-generation study where this effect can be link to body weight effects. No histopathological effects were observed.	No evidence for adversity	T
		Dog	1-year	Oral	1350 ppm	No effects at the highest dose			
		Mouse	6 weeks	oral	4000 ppm	No effects at the highest dose			
		Rat	28 days	oral	5000	No effect at the highest dose			
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		rat	1-generation	oral	800	Increase of the relative weight only (not absolute)			
	Thyroid (histopathology)	Dog	90 days	Oral	1350 ppm	No effects at the highest dose			
		Dog	1-year	Oral	1350 ppm	No effects at the highest dose			
		Mouse	104 weeks	oral	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	90 days	Oral	1000 ppm	No effects at the highest dose			
		Rat	28 days	Dermal	1000 mg/kg bw/d	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	2 years	Oral	2000 ppm	No effects at the highest dose			
		Rat	1-generation	Oral	1600 ppm	No effects at the highest dose			
Evidence of general toxicity	see table on EAS modalities	See table on EAS modalities					See table on EAS modalities	See table on EAS modalities	

Table 5: Lines of evidence for ED activity and adversity after dimethomorph exposure in aquatic vertebrates.

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposures	Effect dose mg/L	Observed effect (positive or negative)	Assessment of each line of evidence	Assessment of integrated line of evidence	Modality
Integrated lines of evidence for endocrine activity									
In *vivo* mechanistic	Male secondary sex characteristics (SSC) in females	Fish / Fathead minnow	FSTRA (3 weeks)	Uptake from water	>0.48	No effect up to and including the maximum tolerated concentration (MTC)	No effect on male SSC in fish	No evidence for endocrine activity in fish	A
	Male secondary sex characteristics (SSC) in males	Fish / Fathead minnow	FSTRA (3 weeks)	Uptake from water	>0.48	No effect up to and including the MTC			E, A, S
	Vitellogenin (VTG) in females	Fish / Fathead minnow	FSTRA (3 weeks)	Uptake from water	>0.48	No effect up to and including the MTC	No effect on VTG in fish		E, A, S
	Vitellogenin (VTG) in males	Fish / Fathead minnow	FSTRA (3 weeks)	Uptake from water	>0.48	No treatment-related effect up to and including the MTC			E, A, S
Integrated lines of evidence for endocrine adversity									
Sensitive to, but not diagnostic of EATS	Abnormal morphology and appearance	Fish / Fathead minnow	FSTRA (3 weeks)	Uptake from water	>0.48	No effect up to and including the MTC	No effect on morphology and appearance in fish	Evidence for adversity (i.e. effects on growth) in combination with general toxicity	n.a.
		Fish / Fathead minnow	ELS (5 weeks)	Uptake from water	>0.92	No effect			
		Fish / Rainbow trout	ELS (14 weeks)	Uptake from water	>0.90	No effect			
	Behaviour	Fish / Fathead minnow	FSTRA (3 weeks)	Uptake from water	>0.48	No effect up to and including the MTC	Weak evidence: Indication in only 1 out of 4 studies at highest tested concentration		
		Fish / Fathead minnow	ELS (5 weeks)	Uptake from water	>0.92	No effect			

		Fish / Rainbow trout	ELS (14 weeks)	Uptake from water	>0.90	No effect			
		Fish / Rainbow trout	21-d toxicity test (3 weeks)	Uptake from water	1.53	Symptoms of intoxication			
	Fecundity	Fish / Fathead minnow	FSTRA (3 weeks)	Uptake from water	>0.48	No effect up to and including the MTC	No effects on reproduction in fish		
	Fertility	Fish / Fathead minnow	FSTRA (3 weeks)	Uptake from water	>0.48	No effect up to and including the MTC			
	Hatching success	Fish / Rainbow trout	ELS (14 weeks)	Uptake from water	>0.90	No effect	No indication		
		Fish/ Sheepshead minnow	ELS (6 weeks)	Uptake from water	>2.01	No effect			
	Length*	Fish / Fathead minnow	FSTRA (3 weeks)	Uptake from water	>0.48	No effect up to and including the MTC	Some evidence: observed in 2 species in 2 out of 3 ELS studies; Effects occur only in combination with general toxicity (see body weight)		
		Fish / Fathead minnow	ELS (5 weeks)	Uptake from water	>0.92	No effect			
		Fish / Rainbow trout	ELS (14 weeks)	Uptake from water	0.24	Decrease			
		Fish / Rainbow trout	21-d toxicity test (3 weeks)	Uptake from water	>1.53	No effect			
		Fish/ Sheepshead minnow	ELS (6 weeks)	Uptake from water	0.266	Decrease			

	Survival of embryos	Fish / Fathead minnow	ELS (5 weeks)	Uptake from water	0.347	Decrease	Inconsistent and weak indication: observed in 1 ELS study only at mid-dose (no statistical significant effect at highest tested concentration); survival at study termination was not affected in any of the chronic fish studies (see below)		
		Fish / Rainbow trout	ELS (14 weeks)	Uptake from water	>0.90	No effect			
General adversity	Body weight	Fish / Fathead minnow	FSTRA (3 weeks)	Uptake from water	>0.48	No effect	Consistent in 2 species and 2 ELS studies.	Overall evidence for adversity (i.e. effects on growth)	n.a.
		Fish / Fathead minnow	ELS (5 weeks)	Uptake from water	>0.92	No effect			
		Fish / Rainbow trout	ELS (14 weeks)	Uptake from water	0.12	Decrease			
		Fish / Rainbow trout	21-d toxicity test (3 weeks)	Uptake from water	>1.53	No effect			
		Fish/ Sheepshead minnow	ELS (6 weeks)	Uptake from water	0.266	Decrease			
	Survival	Fish / Fathead minnow	FSTRA (3 weeks)	Uptake from water	>0.48	No effect	No effect on survival		
		Fish / Fathead minnow	ELS (5 weeks)	Uptake from water	>0.92	No effect			
		Fish / Rainbow trout	ELS (14 weeks)	Uptake from water	>0.90	No effect			

		Fish / Rainbow trout	21-d toxicity test (3 weeks)	Uptake from water	>1.53	No effect			
		Fish/ Sheepshead minnow	ELS (6 weeks)	Uptake from water	>2.01	No effect			

* It is unclear why “length” is classified as “Sensitive to, but not diagnostic of EATS” in Appendix E whereas “body weight” is considered as an indication of general adversity. These parameters are highly correlated and are both used to measure effects on growth which can be caused by all kinds of toxicities.

Table 6: Lines of evidence for adverse effects after dimethomorph exposure in Level 4 studies with birds.

Grouping	Line(s) of evidence	Species	Exposure weeks	Route of exposures	Effect dose ppm	Observed effect (positive or negative)	Assessment of each line of evidence	Assessment of integrated line of evidence	Modality
Sensitive to, but not diagnostic of EATS	Cracked eggs	Birds / quail	1-gen reproduction (24 weeks)	Oral, dietary	>800	No effect	No effect on cracked eggs	No effect on reproduction of birds	n.a.
		Birds / mallard	1-gen reproduction (24 weeks)	Oral, dietary	>800	No effect			
	Egg production	Birds / quail	1-gen reproduction (24 weeks)	Oral, dietary	>800	No effect	No effect on egg production		n.a.
		Birds / mallard	1-gen reproduction (24 weeks)	Oral, dietary	>800	No effect			
	Egg viability	Birds / quail	1-gen reproduction (24 weeks)	Oral, dietary	>800	No effect	No effect on egg viability		n.a.
		Birds / mallard	1-gen reproduction (24 weeks)	Oral, dietary	>800	No effect			
	Eggshell thickness	Birds / mallard	1-gen reproduction (24 weeks)	Oral, dietary	>800	No effect	No effect on eggshell thickness		n.a.
		Birds / quail	1-gen reproduction (24 weeks)	Oral, dietary	>800	No effect			
	Hatchability	Birds / quail	1-gen reproduction (24 weeks)	Oral, dietary	>800	No effect	No effect on hatchability		n.a.
		Birds / mallard	1-gen reproduction (24 weeks)	Oral, dietary	>800	No effect			

Evidence of general toxicity	Body weight	Birds / quail	1-gen reproduction (24 weeks)	Oral, dietary	>800	No effect	No effect on growth	No evidence for general adversity in birds	n.a.
	Body weight	Birds / mallard	1-gen reproduction (24 weeks)	Oral, dietary	>800	No effect			n.a.

2.3. Analysis of the evidence.

The entire data package can be considered complete to meet the data requirements of Regulation 1107/2009 and to have potentially studied adequately EATS modalities under that Regulation. However, in § 3.4.1 of the new ECHA / EFSA ED GD "Sufficient data set for EATS-mediated adversity to support a conclusion on absence of EATS-mediated adversity" it is stated that "*To have the EAS mediated adversity with regards to human and mammals (as non-target organisms) sufficiently investigated, all the data requirements of the specific Regulations, must be fulfilled. This should include all the "EAS-mediated" parameters foreseen to be investigated in an extended one-generation reproductive toxicity study (EOGRTS, OECD TG 443; with cohort 1a/1b including the mating of cohort 1b to produce the F2 generation (OECD 2012b)) or a two-generation reproductive toxicity study (OECD TG 416; test protocol according to the latest version of January 2001 (EOCD 2001)*".

In the case of dimethomorph, the 2-generation study was performed before the implementation of the new 2001 guideline and therefore do not capture all the EAS parameters that should be investigated. The EOGRTS was performed just after the publication of the guideline, cohort 1a/1b were included but the mating of cohort 1b to produce the F2 generation was not performed.

It should be noted that, in the particular case of dimethomorph, investigation of the F2 generation is crucial to determine the adversity of the effects observed, therefore the lack of the F2 generation including EAS modalities can be considered as a data gap (see conclusions below).

According to ToxCast and Tox21 chemical screening programs, dimethomorph was tested in 54 ToxCast Assays related to EATS modalities and the reported limit of cytotoxicity was 2.12 µM. Dimethomorph was not active in any androgen receptor (AR), estrogen receptor (ER), thyroid receptor (TR), or aromatase assays.

Some activity was observed in two out of 20 steroidogenesis assays and is discussed in the following section on Steroidogenesis.

2.3.1. Assessment of the evidence for human health

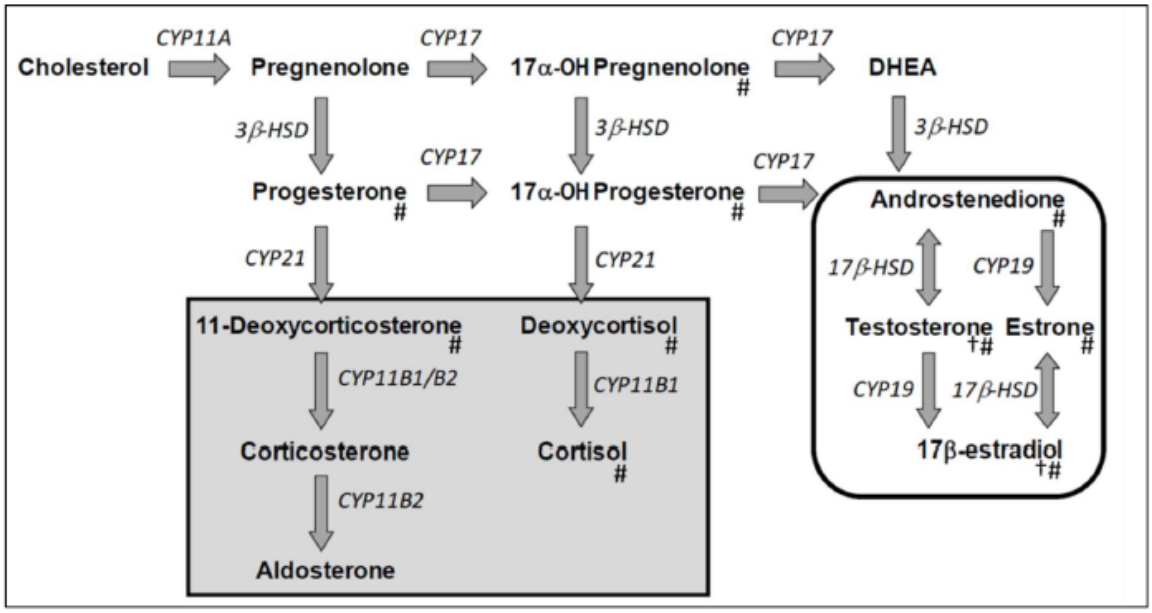
2.3.1.1. EAS modalities

2.3.1.1.1. Evidence for endocrine activity from the *in vitro* mechanistic data

Dimethomorph was inactive in 15/15 ToxCast assays of the estrogen receptor, inactive in 11/11 assays of the androgen receptor, inactive in 3/3 aromatase assays and inactive in 18/20 of the steroidogenesis assays.

The two positive steroidogenesis assays were upregulation of estrone and estradiol. According to the steroidogenesis pathway presented in figure 3, upregulation of these two hormones can be related to aromatase effect of to a direct effect on adrenals. As no effects were observed in aromatase assays and as no in vivo effects were observed on adrenals in all performed studies *in vivo* (except an increase of adrenal weight in a 28-day study in rat at the highest dose tested), these two positive hits are considered to be incidental and not biologically relevant.

Source: OECD (https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-tg456-2011-508.pdf)



#: Hormone assessed in ToxCast/Tox21 steroidogenesis assays,
†: Hormone specified in OECD Test Guideline 456

Fig. 4: Steroidogenesis pathway.

In *in vitro* studies, some effects related to anti-androgenicity have been reported for dimethomorph in five assays (3 YAS and 2 MDA-kb2 assays). Positive results in YAS and MDA-kb2 assays indicates a potential of dimethomorph for binding to the Androgen Receptors (AR) and subsequently a possible antagonism with natural hormones. This occurred at relatively high concentrations *in vitro* and with a steep slope in the dose-dependent response indicating a small concentration range of AR antagonistic activity.

Two assays *in vitro* on estrogen receptors were clearly negative.

In conclusion, the mammalian *in vitro* screening assays showed that dimethomorph does not interfere with the E- or S -modality but they provided some evidence for anti-androgenic activity of dimethomorph.

To address the relevance of these *in vitro* findings, analysis on "EAS-mediated" and "sensitive-to-but-not-diagnostic-of-EAS" is assessed below according to the ED guideline.

2.3.1.1.2. EAS-mediated parameters

The assessment of the *in vitro* mechanistic data on EAS-related activity of dimethomorph showed that only A-mediated parameters were affected. Therefore, parameters that could be potentially related to an anti-androgenic activity of dimethomorph are discussed in the following section.

- **Prostate (weight and histopathology)**

In dogs, slight effects were observed on the prostate in the 90-day and the one-year study. These effects were limited to the highest tested dose of 1350 ppm and consisted of prostate weight reduction (absolute and relative) associated with increased severity of prostatic interstitial fibrosis. It should be noted that prostatic interstitial fibrosis is a common finding in dogs, seen in all groups including controls in the 1-year study.

Histopathological findings were graded minimal (Grade 1), mild (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence.

Only minimal to moderate effects were observed in the study, see table 7

Table 7: Individual data on severity:

Group	animal #	Prostate weight [g]	Prostate Histopathological findings	Grade	Mean severity /group
1	1	8.87	minimal interstitial fibrosis, mild acinar enlargement	1	1.5
	2	9.43	minimal interstitial fibrosis, minimal acinar enlargement	1	
	3	4.72	mild interstitial fibrosis, minimal acinar enlargement	2	
	4	9.98	mild interstitial fibrosis, mild acinar enlargement	2	
2	9	10.91	mild interstitial fibrosis, mild acinar enlargement	2	2
	10	10.82	moderate interstitial fibrosis, mild acinar enlargement	3	
	11	8.61	mild interstitial fibrosis, minimal acinar enlargement	2	
	12	5.54	minimal interstitial fibrosis, mild acinar enlargement	1	
3	17	4.65	minimal interstitial fibrosis, minimal acinar enlargement	1	2
	18	7.1	mild interstitial fibrosis, mild acinar enlargement	2	
	19	6.22	mild interstitial fibrosis, moderate acinar enlargement	2	
	20	4.21	moderate interstitial fibrosis, mild acinar enlargement	3	
4	25	3.57	moderate interstitial fibrosis, minimal acinar enlargement	3	2.5
	26	6.95	minimal interstitial fibrosis, mild acinar enlargement	1	
	27	2.69	moderate interstitial fibrosis, mild acinar enlargement	3	
	28	3.85	moderate interstitial fibrosis, mild acinar enlargement, chronic inflammation minimal	3	

According to the study director, "*The light microscope appearance of the prostates all lay within the normal range for young laboratory dogs. There was an apparent increase in the proportion of interstitial fibrosis (including smooth muscle) as the group dosages increased. However, some of this effect was probably due to a slightly different level of section and in none of the animals was the degree of interstitial tissue of a pathological nature*." (Excerpt of the discussion in the results section of the report (Page 72)).

Conclusion: there is a significant reduction in prostate weight after 52 weeks of treatment, but no clear histopathological correlation could be observed.

In rat, prostate weight was investigated in 2 studies. No effects were observed in the 28-day dermal study in rats. A slight decrease of the absolute prostate weight was observed at the high dose of parental animals in the extended 1-generation, but this change was within historical controls data and there was no histopathological correlate. In addition, the relative prostate weight did not show a significant decrease. Therefore, the decrease of prostate weight was related to the slightly but not significantly reduced terminal body weight (-4%) in this test group.

Overall, prostatic effects, mainly seen in dogs, are common in aging animals, no clear evidence of histopathology effects were observed in association with organ weight decrease. No effects on prostate were observed in rats. In conclusion, this effect cannot be clearly link to an endocrine mode of action.

- **Testis (weight and histopathology)**

Effects on testes weight were investigated in 2 studies in dog and 7 studies in rats.

In dog, in the 1-year study, statistically significant increases in testes-to-body weight ratios were observed at 450 and 1350 ppm without any histopathological findings. Absolute testes weights were not affected. This effect was not seen in the 90-day study.

In rats, one out of seven study showed effect on testicular weight. Only a slight change in testes weight of the 1a and 1b cohort was observed in the extended 1-generation without histopathological correlate. The testes weights were significantly increased in test groups 12 (relative) and 13 (absolute and relative). There was no comparable effect in F0 generation parental males. In Cohort 1A males, the relative testes weights were increased in test groups 02 and 03 but there was no histopathological correlate and the increased testes weights could be related to reduced terminal body weights. Therefore, a treatment-related effect seemed rather unlikely.

Effects on tests histopathology were investigated in 2 dog studies and 9 rat studies. No changes were observed in dogs. In rats, no changes were observed in mid-term and reproductive studies. In the long-term rat study (chronic and carcinogenicity) an increased incidence of testicular interstitial cell adenomas was observed in treated groups. This effect was also observed in controls. This effect is not considered as adverse for the following reasons:

(1) Increased longevity in the chronic toxicity study (see Table 8)
For the 2-year chronic toxicity study, increased longevity observed for males at 2000 ppm likely predisposed these animals to increased incidences of benign interstitial cell tumors given that more animals in this group lived longer than animals in the other treatment groups or control group. Specifically, the survival rate at 104 weeks for males at 2000 ppm was 75%, as compared to 45, 65, and 70% for males at 0, 200, and 750 ppm, respectively. For premature male decedents, only one rat per each dietary concentration had a benign interstitial cell tumor. All other testicular tumors of this cell type were observed in males that survived until terminal sacrifice. In the 104-week carcinogenicity study the survival rate of male animals of the control group was also lower than the survival rates of male animals in the treatment groups. However, the differences are lower than in the chronic toxicity study.

Table 8: Mortality in male rats receiving dimethomorph for 104 weeks

Interval (week)		**Dose level (ppm)**		
	0	**200**	**750**	**2000**
Chronic toxicity study				
0 – 52	1/20 (5)[a]	1/20 (5)	0/20 (0)	0/20 (0)
53 – 78	3/19 (16)	2/19 (11)	0/20 (0)	2/20 (10)
79 – 104	7/16 (44)	4/17 (24)	6/20 (30)	3/18 (17)
0 - 104	11/20 (55)	7/20 (35)	6/20 (30)*	3/20 (25)
Carcinogenicity study				
0 – 52	1/50 (2)	0/50 (0)	3/50 (6)	2/50 (4)
53 – 78	5/49 (10)	6/50 (12)	7/47 (15)	5/48 (10)
79 – 104	22/44 (50)	14/44 (32)	6/40 (15)	16/43 (37)
0 - 104	28/50 (56)	20/50 (40)	16/50 (32)	23/50 (46)
Range historical mortality (0 – 104 weeks) 30 – 56 %				

a: the number in bracket presents % mortality, * $p < 0.05$

(2) No statistically significant oncogenic effects (see Table 9)
There was no statistical significance in the incidence of testicular interstitial cell adenomas between the control group and any of the dimethomorph treated groups for either study ($p > 0.05$). Moreover, in a trend analysis, considered to be a more sensitive test to detect dose-related trends than a pair-wise comparison, no statistically significant positive trends were observed ($p > 0.05$).

Table 9: Incidence of testicular interstitial cell adenoma in male rats receiving dimethomorph for 104 weeks

		Dose level (ppm)		
	0	**200**	**750**	**2000**
Chronic toxicity study				
	2/19[a] (10.5)[b]	5/20 (25)	4/20 (20)	6/20 (30)
Trend test p* 0.14				
Carcinogenicity study				
	5/50 (10)	7/49 (14)	8/50 (16)	10/50 (20)
Trend test p* 0.11				

*p values reported in this table were from Peto analysis
a number of animals with IC adenoma/number of animals examined
b number in bracket is percent value

(3) Incidence values for benign testicular interstitial cell tumors are within or just slightly higher the historical ranges for the testing laboratory (see Table 10). The range of the historical spontaneous incidence of testicular interstitial cell adenomas for carcinogenicity studies performed at Inveresk Research International (IRI), the laboratory which conducted the rat carcinogenicity study on dimethomorph, is from 4 % (minimal control incidence) to 20 % (maximal control incidence). Therefore, the 20 % incidence of interstitial cell adenoma of the testes in the high-dose group from the rat carcinogenicity study with dimethomorph is within the range of the historical control incidence of this laboratory. In addition, the incidence from the combined two studies for interstitial cell adenoma of the testes in the high-dose group is 16/70 (22.9%), just slightly higher the maximum historical incidence.

Table 10: Historical incidence of neoplastic and focal hyperplastic lesions in testicular interstitial cells of control Sprague-Dawley rats in 104-week studies at Inveresk Reseach International

Study I.D.	**Date**	**No examined**	**Incidence ***		
			Adenoma	**Hyperplasia**	**Combined**
198	1986-1988	50	10 (20)[a]	0	10 (20)
507	1987-1989	50	9 (18)	1 (2)	10 (20)
827	1986-1988	48	4 (8.3)	9 (18.8)	13 (27.1)
261	1985-1987	49	2 (4.1)	7 (14.3)	9 (18.4)
717	1985-1987	48	5 (10.4)	3 (6.2)	8 (16.7)
671	1984-1986	50	6 (12)	1 (2)	7 (14)
729	1984-1986	50	4 (8)	0	4 (8)
802	1984-1986	50	6 (12)	0	6 (12)
Range			4.1 – 20 %	0 – 18.8 %	8 – 27.1 %

*There was no single case of malignant tumors recorded in these studies
a Numbers in bracket represents incidence in %

(4) No progression of benign testicular adenomas to malignant carcinomas
There was no evidence of progression of benign interstitial cell adenomas to malignant interstitial cell carcinomas.

(5) No decreased latency for the benign testicular interstitial cell tumors from the high-dose group versus the controls.
There was no indication that testicular interstitial cell adenomas developed earlier in treated rats, as compared to controls (see Table 11).

Table 11: Observation of testicular IC tumors in premature decedents in male rats receiving dimethomorph

			Dose level (ppm)		
	0		200	750	2000
		Chronic toxicity study			
week	1/10[a] 94[b]		1/7 102	1/6 99	1/5 92
		Carcinogenicity study			
week	1/28 87		0/19	1/16 91	3/23 75, 100, 101

a number of animals with tumor/number examined
b week of study when incidence IC tumors were found

(6) Lack of oncogenic effects for the testes in the 2-year oncogenicity study in the mouse

(7) Absence of mutagenic or genotoxic activity of dimethomorph

(8) Very conservative size criterion was used on microscopic examination to diagnose benign testicular interstitial cell adenomas. Thus, some of these benign tumors may be downgraded to interstitial cell hyperplasia. The major criterion used for the differential diagnosis of benign testicular interstitial cell adenoma versus hyperplasia was based on size. The pathologists considered that any focal, expansible aggregates of interstitial cells of greater diameter than an average arteriole but smaller than a cross section of a seminiferous tubule were recorded as focal hyperplasia. Those greater in diameter than an average cross section of seminiferous tubule were classified as an adenoma. However, less conservative, present day diagnostic criteria for standardization purposes recommend that the diameter (or cross sections) of 3 or more seminiferous tubules be set as the arbitrary separation of interstitial cell neoplasia, as distinguished from focal hyperplasia. Thus, some of the benign interstitial cell adenomas may possibly downgraded to focal interstitial hyperplasia, using the present-day, less conservative size criterion.

Overall conclusion on testes effects:

Effects seen on testes weight observed in some studies were not associated with histopathological lesions. In the chronic rat studies, incidence of testicular tumors in treated groups can be explain by multiple factors and this effect is therefore not considered to be substance related.

Endocrine mode of action can therefore be excluded for testes effects.

- **Age at preputial separation**

Delayed in preputial separation was observed in pups of the mid- and high-dose of the extended 1-generation study. This delay in sexual maturation was only seen in males. This effect may be partly explained by the decreased body weight leading to slow general development.

When compared to body weight, it is clear that for the mid dose (figure 4), this effect is not relevant, for the high dose, the shift in the right of treated animals observed in figure 5 show a slight treatment related effect.

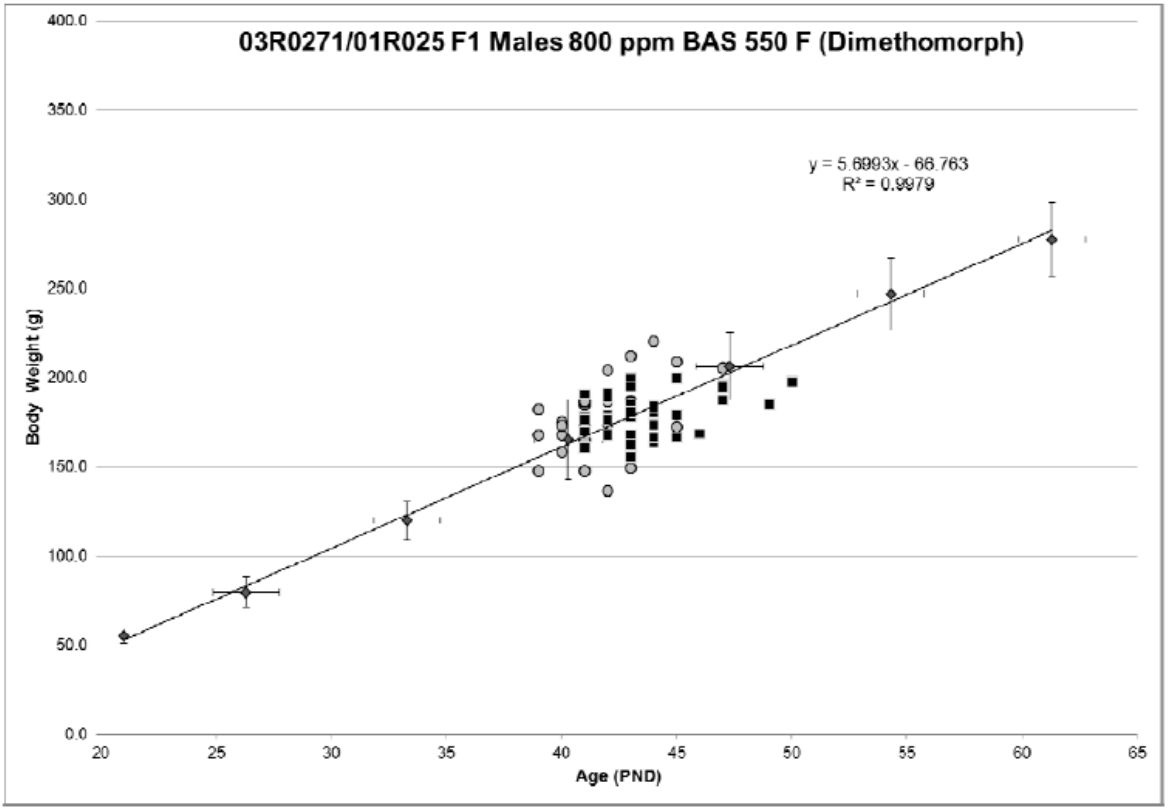


Fig. 5: Sexual maturation time compared to body weight (round point: controls, square point: treated animals)

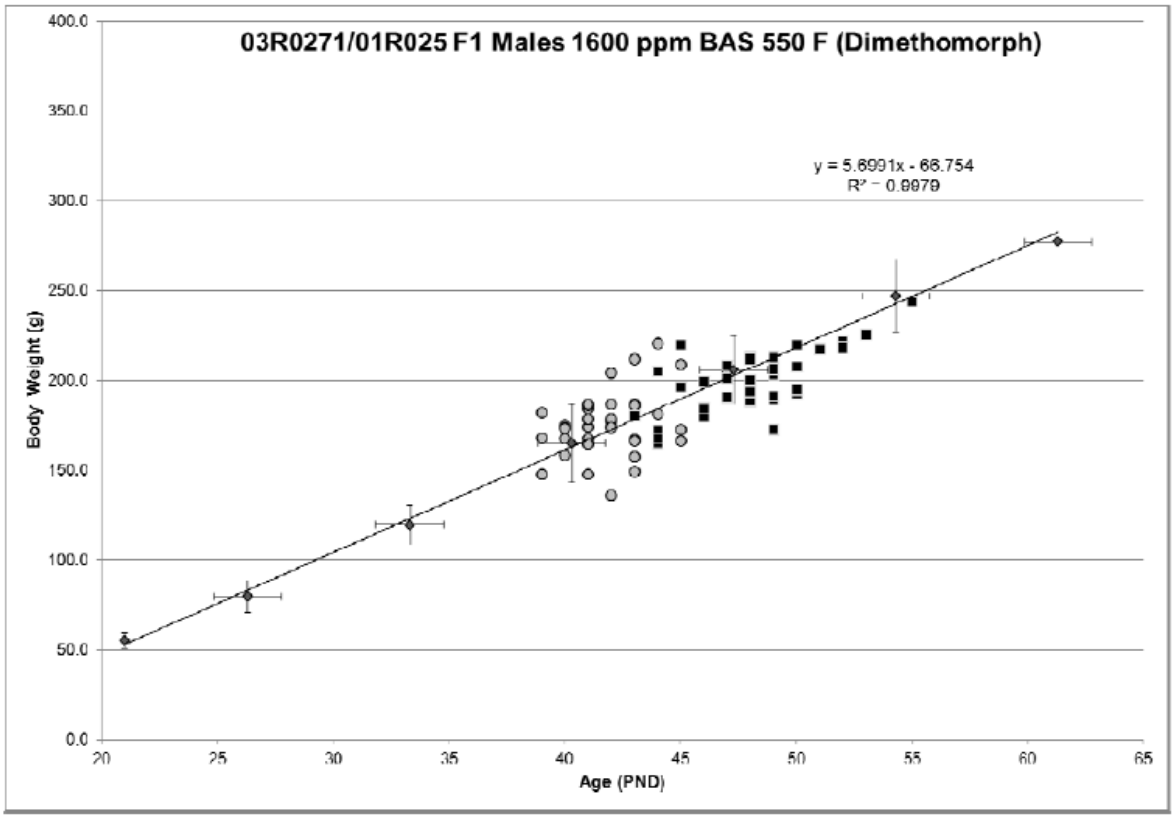


Fig. 6: Sexual maturation time compared to body weight (round point: controls, square point: treated animals)

A mechanism of action involving anti-androgenicity cannot be completely ruled out for effects observed in the high dose.

- **Anogenital distance/Anogenital index (AGD/AGI)**

A slight effect on anogenital distance/index in mid and high dose males was observed and may be partly explained by the decreased body weight leading to slow general development.

Table 12: Anogenital distance and index of lactation pup on PND1 litter based

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
PND 1								
male pup litter nb	N = 24		N = 23		N = 25		N = 22	
AG distance [mm]	3.16	0.13	3.10	0.19	**2.99****	0.16	**2.87****	0.21
historical control [mm]	2.99–3.15 (mean 3.06 sd 0.04)							
AG index	1.65	0.04	1.62	0.09	**1.59****	0.05	**1.57****	0.09
historical control	1.58–1.67 (mean 1.62 sd 0.03)							

* = p<0.05; ** = p<0.01; Dunnett test (two-sided)

AGD and AGI in male of the 800 ppm dose in within the historical controls when considering the calculation of the values on a litter base.

Only the changes at the top dose are statistically significant and slightly outside the historical controls.

It must be noted that AGD of controls of the study is higher than historical controls. This finding can explain partly the statistical significance observed in the mid dose.

A mechanism of action involving anti-androgenicity cannot be completely ruled out for effect observed in the high dose.

- **Other EAS-mediated endpoints**

No other changes were observed in other EAS endpoints. No effects at all were observed in females in all studies.

In males, effects like Coagulating gland histopathology, Epididymis histopathology, Genital abnormalities, male mammary gland histopathology, seminal vesicles histopathology, nipple development, and sperm parameters were not affected.

2.3.1.1.3. Parameters sensitive to but not diagnostic of EAS

- **Adrenal (weight and histopathology)**

No effects on adrenal were observed in rats and dogs (except in one rat study a change of adrenal weight was observed, as this effect was not reproducible in other studies and was not correlate with histopathology finding, it is considered as a chance finding).

- No effects on fertility were observed.

- **Gestation length**

A slight effect on gestation length was observed in the extended 1-generation at the top dose. This effect was not observed in the 2-generation study.

In the extended 1-generation study, the mean duration of gestation values varied between 22.0 and 22.3 days in control, 300 and 800 ppm test groups without any relation to dosing. The mean duration of gestation was statistically significantly decreased in comparison to the concurrent control group in the 1600 ppm test group ($p \leq 0.01$). The value (21.4 days) was marginally below the historical control range (21.5 to 22.3 days), while the concurrent control (22.3 days) was at the upper range of the historical control data.
When compared to individual data, gestation duration is between 21 and 23 days (as it was also observed in the 2-generation study). Moreover, for the gestation length the intrinsic vagueness of the measurement has to be considered. Gestation length is measured from the detection of sperm after each mating to the day of parturition. For detection of sperm this means the female is placed in the cage of the male partner at 16:00 pm and the next check for sperm is taking place around 7.00-9.00 am of the following morning, thus, mating may have taken place within a time span of 17-19 hours. Similarly, animals are checked for parturition twice daily on weekdays and the day of parturition is given as 24h period from about 15:00 of one day until about 15:00 of the following day, again adding an uncertainty of about 12 hours. Considering the low accuracy of the measurement, the marginal difference of 0.1 day compared to the historical control range observed for the high dose can be considered as very minor.
Based on the slight difference between high dose group and historical controls and taking into account that concurrent controls were in the upper range of the historical controls and, moreover, based on the fact that no effects were seen in the 2-generation study with the appropriate statistical method, this slight decrease in duration length can be considered as a chance finding. According to the study director, as this effect didn´t go along with any adverse effects on developmental status and well-being of the newborns, it is considered to be non-adverse and of no toxicological relevance.

- **Pup development**

In the extended 1-generation pup development was affected (decrease body weight and body weight gain in the mid-and high dose). This effect is considered to be a sign of systemic toxicity and not related to an endocrine mode of action.

In rabbit developmental studies, some effects were observed regarding pup viability, pre- and post-implantation loses. No malformations were observed. These effects were correlated with abortion of pregnant females as a consequence of severe systemic toxicity. Therefore, this effect is not considered to be endocrine-mediated.

- **Other parameters sensitive but not diagnostic of EAS**

No relevant effects on the following reproduction-related parameters were observed: fertility, birth index and pup survival index, dystocia, foetal development, abnormalities, lactation index, litter size and viability, number of implantations, sex ratio, corpora lutea, number of ovarian follicles.

2.3.1.2. T modality

Dimethomorph was inactive in 6/6 ToxCast assays of the thyroid receptor. No effects were noted in thyroid or thyroid hormone levels that might suggest disruption of thyroid function. Only a slight effect on absolute thyroid weight were observed in the extended 1-generation but not on relative weight and without histopathology), this effect is considered to be due to general toxicity (body weight changes and decreased body weight gain). There is strong weight of evidence (no effect in ten studies measuring thyroid weight and/or histopathology) that dimethomorph has no effect on the thyroid modality.

In summary, dimethomorph does not interfere with the E, S or T modality but there is some evidence for anti-androgenic activity and adverse effects potentially caused by this activity. Therefore, a mode of action (MoA) analysis for the A-modality is performed.

Summary of the ED assessment of dimethomorph for the EATS modalities for human health following ECHA / EFSA ED GD (2018) (see Figure 1 "Flowchart illustrating the ED assessment strategy on page 13):

1) Have the EATS-mediated parameters been sufficiently investigated?

Note: *To have the EAS-mediated adversity with regard to humans and mammals (as non-target organisms) sufficiently investigated (…) all the 'EAS-mediated' parameters foreseen to be investigated in an extended one-generation reproductive toxicity study (EOGRTS; OECD TG 443; with cohort 1a/1b including the mating of cohort 1b to produce the F2 generation (OECD, 2012b)) or a two-generation reproductive toxicity study (OECD TG 416; test protocol according to latest version of January 2001 (OECD, 2001))* (see ECHA EFSA ED GD (2018), p. 31)

- **Not sufficiently investigated within current relevant and available information** (i.e. not all parameter foreseen in the current version of OECD TG 443 were investigated)

2) Has the EAS-mediated adversity been observed?

- **There is no clear evidence of EAS-mediated adversity in available mammalian dataset**

→ **Scenario 2a**

3) Has EAS-related activity been observed?

- **Some evidence for A-mediated activity in mammals**

→ **Scenario 2a (i)**

Conclusion: a MoA analysis is required for the A-modality

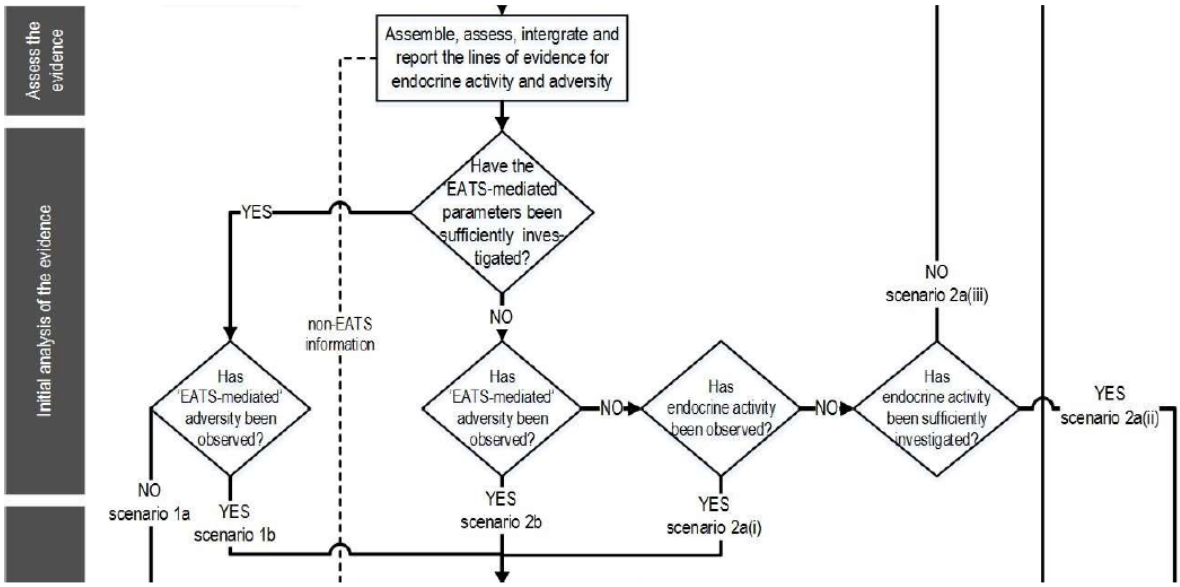


Fig. 7: Excerpt of the flowchart shown on page 13 of the ECHA/EFSA Guidance Document

2.3.2. Assessment of the evidence in wild mammals

While for mammals as non-target organisms the same dataset on endocrine activity from *in vitro* assays and *in vivo* studies is used as for to the toxicological assessment, the determination of adverse effects will be a key difference to toxicology, since for wild mammals the protection goal is at the population level. Population relevant effects are considered those affecting survival, reproduction, growth and development (EC 2011). However, there is no official guidance with a detailed description of endpoints related to population relevant effects. In the EU regulatory context, neither the ECHA/EFSA ED GD (2018) nor the current guidance document on the Risk Assessment for Birds and Mammals (EFSA, 2009) provides a detailed description of which measured endpoints in mammalian laboratory studies are considered population relevant. In the absence of official regulatory guidance, we follow the proposals of a recent publication on the relationship of individual-level effects and potential population-relevant effects in the context of the evaluation of endocrine active substances (Marty et al., 2017). Primary apical endpoints of general importance for population relevance (not necessarily ED related) are body weight, growth, reproductive success, offspring viability, development and potentially behavioural endpoints (see Table 1, Marty et al., 2017). This publication provides in its Table 1 a detailed description of the primary endpoints of relevance for endocrine assessment measured in ecotoxicology and mammalian toxicology studies and points out at endpoints of population relevance. To facilitate the understanding of key information from this paper, the following table (Table 13) summarizes the population relevant effects according to Marty et al. (2017).

Table 13: *In vivo* laboratory tests and population-relevant endpoints for mammals proposed by Marty et al. (2017, Table 1) in the context of the ecotoxicological assessment of endocrine-active substances

Test type	Primary measured endpoint- Population relevant
-Repeated dose (oral toxicity in rodents and non-rodents) -Carcinogenicity -Chronic toxicity	-Body weight
-Reproductive or developmental toxicity screening test -Combined repeated dose toxicity with reproduction or developmental toxicity screening text	-Parental body weight -Offspring body weight -Behaviour* -Reproductive success -Offspring survival
-Prenatal development	-Body weight -Development stage
-Developmental neurotoxicity	-Body weight -Behaviour*
-Extended 1-generation reproduction test	-Reproductive success
-2-generation reproduction test	-Parental body weight -Offspring body weight -Behaviour* -Offspring survival

*Potentially population-relevant

The assessment for wild mammals as non-target organisms focuses on the question whether dimethomorph fulfils the ED criteria with emphasis on their relevance at the population level following the endpoints proposal by Marty et al. (2017).

The database and conclusions for dimethomorph are described in detail in the evaluation conducted by toxicologists for the human assessment (see above). The toxicological evaluation includes full details supporting that there are no 'E', 'S' and 'T' mediated adverse effects by dimethomorph in the mammalian studies evaluated. Therefore, these three modalities do not require further evaluation for non-target mammals. The assessment carried out by the toxicologists concludes that the only modality that may potentially be affected after exposure to dimethomorph is 'A' (anti-androgenicity). Therefore, the assembling and analysis of evidence for non-target mammals focuses on this modality only. The following three slight *in vivo* effects were observed in the pups of the extended 1-generation study (see chapter 2.3.1.1.2): changes in anogenital distance (AGD)/index (AGI) of males at the high dose (1600 ppm), delayed sexual maturation of males in the high dose and decreased seminal vesicle weight at the high dose. Notably, these effects are observed in the presence of systemic toxicity (general growth retardation).

The effects related to dimethomorph are not considered population relevant for wild mammals as none of the potentially endocrine sensitive parameters related to fertility, reproduction and development of offspring were adversely affected in the one-generation reproductive toxicity study (EOGRTS) or the 2-generation reproduction study following OECD TG 416.

According to Commission Regulation 2018/605 amending the Plant Protection Regulation No. 1107/2009 and based on the existing dataset, dimethomorph cannot be considered as having endocrine disrupting properties in non-target mammals as it does not show adverse effects impairing functional capacity that are considered relevant at the population level, and which are a consequence of an endocrine mode of action.

However, it should be noted that, in the particular case of dimethomorph, toxicology concluded that the investigation of the F2 generation is crucial to determine the adversity of the effects observed and a new extended 1-generation study with additional cohorts (especially a second-generation cohort as mentioned in the ED guidance) is proposed (see also chapter 2.5.1). Therefore, a final conclusion on the ED properties for wild mammals needs to consider the data from this proposed new study. At this point, no further study is proposed to specifically address potential ED properties of dimethomorph in wild mammals.

2.3.3. Assessment of the evidence in non-target vertebrates other than mammals

2.3.3.1. Fish (EAS-modalities)

The chronic fish data package of dimethomorph consist of three early life-stage (ELS) studies (OECD TG 210) on three different fish species, one 21-d assay with rainbow trout (OECD TG 204), and a fish short term reproduction assay (FSTRA, OECD TG 229) to determine potential effects of dimethomorph on reproduction and endocrine-relevant biomarker endpoints in fathead minnow (*Pimephales promelas*) (see dimethomorph dossier, M-CA 8.1).

The fish ELS test (OECD TG 210) is classified as a CF level 4 study and designed to identify the chronic lethal and sub-lethal effects of chemicals in fish early life stages. The ELS test gives information on general adversity that can help with the interpretation of data for ED identification. It includes parameters that might be sensitive to, but not diagnostic of EATS modalities such as hatching success and development. The FSTRA (OECD TG 229) is classified as a CF level 3 test and was primarily designed as a screen for *in vivo* ED activity in fish, but it also provides some information on adverse effects on fecundity. The FSTRA with the fathead minnow covers *in vivo* mechanistic and EATS-mediated parameters that are indicative of the E, A, and S modalities: vitellogenin (VTG) and male secondary sex characteristics (i.e. number of tubercles). In addition, the FSTRA covers non-specific apical parameter including reproduction, that may respond to endocrine perturbation. Thus, *in vivo* mechanistic, EAS-mediated, and population-relevant endpoints (i.e. growth and reproduction) were analysed in the available chronic fish studies (ELS, FSTRA) with dimethomorph.

In the FSTRA with dimethomorph fish were exposed for 21 days to a dilution water control and to three test item concentrations of 0.047, 0.15 and 0.48 mg a.s./L (spacing factor of 3). The highest exposure concentration was equal to the maximum tolerated concentration (MTC), i.e. the test concentration before systemic toxicity occurs (see Wheeler et al. 2013) to optimize finding relevant endocrine-mediated effects should these occur. The survival rate of males and females was 100% in all treatment groups. No statistically significant deviations in fish wet weight and total length compared to the control were observed at any test concentration. No effects on VTG levels in female fish occurred. A statistically significant increase of the VTG concentration in male fish the intermediate, 0.15 mg a.s./L, test group was indicated by one of the two applied statistical tests. However, the median vitellogenin values of the males were not dose-dependently altered. Furthermore, male VTG levels are inherently more variable then female VTG levels, much lower and often close to the limit of quantification, causing further analytical variability. The values of female fish were of lower variability and were not statistically significantly different from the control group in any of the test substance groups. Therefore, the statistically significant higher values of the VTG concentration in males in the intermediate test group were considered as incidental and not treatment-related. The fertilization success (i.e. percentage of fertilized eggs) in the control and all test item concentrations was 99.8%. The fecundity of treated fish remained unaffected, the mean number of eggs per female per day was not statistically significantly different in comparison to the control group. Tubercles were not observed in females. For males, no statistically significant differences in mean tubercle scores compared to the control ocurred in any test concentration. No symptoms of toxicity regarding behaviour, coloration/banding, changes in ovipositor appearance or size of dorsal nape pad were observed. Histopathological samples of the gonads were not evaluated, since the results did not indicate a necessity of this evaluation for the interpretation of the results.

Table 14: List of parameters investigated in the fish short-term reproduction assay (FSTRA, OECD TG 229) with dimethomorph. No treatment-related effects on any of the parameters occurred at or below the maximum tolerated concentration (MTC).

Parameter name	Parameter group	Indicative of	Observation
Vitellogenin (VTG) in females	*In vivo* mechanistic	E, A, S	No effects up to and including the maximum tolerated concentration (MTC)
Vitellogenin (VTG) in males	*In vivo* mechanistic	E, A, S	
Male SSC in females (Tubercles)	EATS-mediated	A	
Male SSC in males (Tubercles)	EATS-mediated	E, A, S	
Behaviour	sensitive to, but not diagnostic of, EATS	N	
Morphological abnormalities	sensitive to, but not diagnostic of, EATS	N	
Fecundity (eggs/female/day)	sensitive to, but not diagnostic of, EATS	N	
Fertiliy (% fertile eggs)	sensitive to, but not diagnostic of, EATS	N	
Length	sensitive to, but not diagnostic of, EATS	N	
Survival	sensitive to, but not diagnostic of, EATS	N	
Body weight	sensitive to, but not diagnostic of, EATS	N	

In summary, no treatment-related effects on diagnostic endpoints (tubercles and VTG) nor apical endpoints (fecundity and growth) were observed over the 21-d exposure period at or below the MTC. Accordingly, the RMS concluded in the dRAR that dimethomorph did not show any (anti-)estrogenic or (anti-)androgenic potential when tested at the MTC in fathead minnow (dRAR for Dimethomorph - Volume 3 B.9 (AS)).

There were no adverse effects in the 21-d assay with rainbow trout (OECD TG 204) except symptoms of intoxication at the highest tested concentration (i.e. 1.53 a.s. mg/L). In the three fish ELS tests (OECD TG 210) with fathead minnow, sheepshead minnow, and rainbow trout, dimethomorph consistently affected growth (i.e. length and body weight), with rainbow trout being the most sensitive of the tested species (LOEC = 0.12 mg/L). In addition, further unspecific effects were inconsistently found in the ELS studies. A decrease in survival of embryos (in fathead minnow at 0.347 mg a.s./L) and an increase of time to hatch (in at 0.266 mg a.s./L in sheepshead minnow) occurred only in one of the three ELS studies. None of the affected parameters is diagnostic of EATS modalities but considered as indication of general adversity and can be caused by all kinds of toxicities. Because dimethomorph did not show any ED-mediated activity in the FSTRA it can be concluded that the general adverse effects in the ELS studies were not caused by endocrine disruption but systemic toxicity of dimethomorph. Thus, dimethomorph does not fulfil the ED criteria for non-target organisms other than mammals in order to be identified as ED in accordance with ECHA/EFSA ED GD (2018).

Following the ECHA/EFSA ED GD (2018), the FSTRA is the preferred test system to sufficiently investigate the EAS modalities for non-target organisms other than mammals. Because a valid and reliable FSTRA is available for dimethomorph, no further data needs to be generated, in accordance with the ECHA/EFSA ED GD (2018), to finalize the assessment of the EAS endocrine potential of dimethomorph in non-target organisms other than mammals.

Summary of the ED assessment of dimethomorph for the EAS modalities in non-target organisms other than mammals following ECHA / EFSA ED GD (2018) (see Figure 1 "Flowchart illustrating the ED assessment strategy on page 13):

1) Have EAS-mediated parameters been sufficiently investigated?

Note: "*To have the 'EAS-mediated' parameters for other non-target organisms sufficiently investigated, the 'EAS-mediated' parameters foreseen to be measured in the Medaka extended one-generation test (MEOGRT, OECD TG 240)) should have been investigated. Alternatively, a FLCTT* (= fish life-cycle toxicity test) *covering the 'EAS-mediated' parameters foreseen to be measured in the MEOGRT is acceptable*" (see ECHA EFSA ED GD (2018), p. 31)

- **Not sufficiently investigated within current relevant and available information** (i.e. no MEOGRT nor FLCTT with dimethomorph is available)

2) Has the EAS-mediated adversity been observed?

- **No evidence of EAS-mediated adversity in non-target organisms other than mammals**

→ **Scenario 2a**

3) Has EAS-related activity been observed? Sufficiently investigated?

Note: "*To consider the E, A, S modalities for non-target organisms other than mammals sufficiently investigated, preferably the 'Fish short term reproduction assay' (FSTRA; OECD TG 229) should have been conducted; however, the 21-day fish assay OECD TG 230 is acceptable as well. If data are already available covering the mechanistic parameters investigated in OECD TG 229 or OECD TG 230 (e.g. OECD TG 234), then those data could be used instead.*"

- **No evidence of EAS-mediated activity in non-target organisms other than mammals**
- **EAS-mediated activity is sufficiently investigated with available information** (i.e. a FSTRA was conducted with dimethomorph)

→ **Scenario 2a (ii)**

Conclusion: Dimethomorph does not meet the ED criteria for the EAS-modalities in non-target organisms other than mammals

2.3.3.2. Amphibians (T-modality)

The ECHA/EFSA ED GD (2018) recommends the Amphibian Metamorphosis Assay (AMA; OECD TG 231) and the Larval Amphibian Growth and Development Assay (LAGDA; OECD TG 241) to sufficiently investigate thyroid-related activity and adversity, respectively, in non-target organisms other than mammals. However, the ECHA/EFSA ED GD (2018) also suggests (when a dossier is already available) to consider the existing data before conducting additional vertebrate tests.

In the case of dimethomorph, there is no indication of thyroid-related activity or adverse effects on the thyroid system neither in the regulatory mammalian studies nor in thyroid receptor binding assays in ToxCast/Tox21 (see section 2.3.1.2). Furthermore, OECD GD 150 cites a review by Pickford (2010) that investigated the extrapolation of thyroid effects between mammalian and amphibian screening models. The review concluded that there seems to be a good foundation for extrapolation of qualitative screening level information between these two animal groups. Against this background, the T-modality does not require further evaluation for non-target mammals and it is reasonable to focus the ED assessment of dimethomorph in non-target organisms on the A-modality, for which activity of dimethomorph was indicated in mammalian endocrine screening *in vitro* assays. Following the ECHA/EFSA ED GD the A-modality is sufficiently addressed for non-target organisms other than mammals with the available data package on dimethomorph (see section above).

In conclusion, considering all available information, adverse effects of dimethomorph on the thyroid system in non-target organisms are not to be expected. Therefore, there is no need for amphibian tests to clarify the potential ED properties of dimethomorph. Additional amphibian studies should not be conducted to avoid unnecessary vertebrate testing (please note that hundreds of animals are required for the amphibian tests).

2.3.3.3. Birds

Dimethomorph was tested in two avian 1-generation studies with bobwhite quail (*Colinus virginianus*) and mallard (*Anas platyrhynchos*). In the standard avian reproduction studies (OECD TG 206), all parameters as detailed in section A of Table 17 of the ECHA/EFSA ED GD (2018), were investigated. Gross-pathological investigations of the parental birds were conducted in both studies. Measurements of biochemical parameters like hormone levels or histopathological examinations of endocrine organs are not part of the guidelines for standard avian reproduction studies and thus were not carried out. No adverse effects were reported for any of the measured endpoints in both available reproduction studies, and the NOEL was confirmed at 800 mg a.s./kg bw, the highest dose tested. The avian reproduction test is classified as a CF (Conceptual Framework) level 4 study whose endpoints are sensitive to, but not diagnostic of EATS (estrogen/ androgen/ thyroid/ steroidogenic) modalities. Therefore, it is not possible to draw a final conclusion on EATS-mediated adverse effects for birds as no single parameter is considered diagnostic for a specific endocrine mediated activity (Table 17, EFSA 2018). However, based on the existing dataset which shows no adverse effects it is considered highly unlikely that dimethomorph fulfils ED criteria in birds.

The assessment strategy for birds is driven by the acknowledgement in the ECHA/EFSA ED GD (2018, section 4.3.2.4) of the limited number of standard *in vivo* methods and the little information that can be obtained on ED-related effects from the standard bird reproduction studies. In the same section, the GD also indicates that more research is required on endocrine and birds. The GD further indicates that the standard studies listed in the GD do not provide enough information to allow for conclusive evidence of endocrine activity or adversity; in fact, data from bird studies are considered on a case-by-case basis as providing complementary information for comparison with the more suitable data from studies with mammals, fish and/or amphibians (ECHA/EFSA ED GD (2018), section 3.3.1).

In line with the technical recommendations in the ECHA/EFSA ED GD (2018) no further avian experimental data should be requested and therefore no further studies with birds are proposed.

2.4. Mode of action (MoA) analysis.

In the assessment of evidence for human and mammals, some *in vitro* activity and *in vivo* effects potentially related to anti-androgenicity were observed. Considering these findings, anti-androgenicity is postulated for the MoA analysis.

2.4.1. MoA analysis for human and mammals.

Considering the adverse outcome pathway (AOP) for androgen receptor (AR) antagonists (Manibusan and Touart, 2017), some evidence is available that dimethomorph can act as an AR antagonist at macromolecular level and organ/system (i.e. effects on AGD and seminal vesicles, delay in sexual maturation) but no evidence of impairment of reproductive capacity have been observed (see Figure 6).

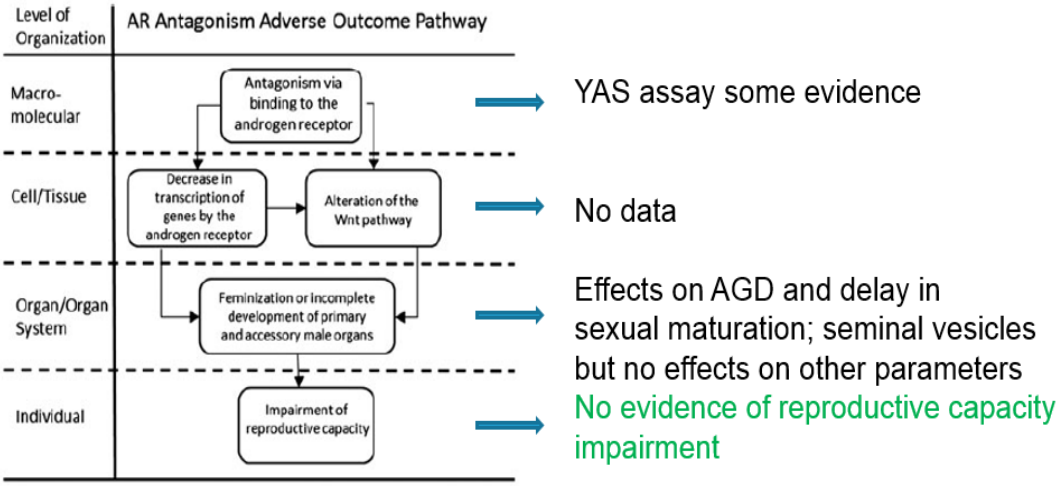


Fig. 8: AOP for anti-androgens

Other typical effects for an anti-androgen substance such as changes in sperm parameters, nipple retention, changes in hormonal levels, histopathological finding in male sexual organs, effects on estrous cyclicity and malformation in male genital organs (i.e. hypospadias) are not observed. Therefore, changes in AGD and delay in sexual maturation could be partly attributed to the delay of male general development (growth retardation) and a strong evidence of endocrine disruption can be excluded.

These effects have been seen in pups at the high dose of the extended 1-generation study. In the 2-generation study no evidence of reproductive capacity impairment was observed at the highest tested dose (1000 ppm). As the highest dose tested in the extended 1-generation was 1600 ppm, there is some uncertainty if these effects on organs/system could lead to reproductive impairment if the pups would be used to produce a second generation. As mention in section 2.3 – analysis of the evidence, a data gap could be identified to draw a firm conclusion on the adversity of these effects.

To clarify the uncertainties related to the effects observed and their adversity on functional capacities (according to the WHO definition reported in the guidance for identifying ED), it is proposed to perform an extended 1 generation study (OECD TG 443) a with specific design to cover the following end-points:

- Potential functional impairment in the second generation due to the developmental effects observed in the first generation at the top dose (AGD/AGI and delay in male sexual maturation)
- Potential impairment of pup neurodevelopment potentially due to a delay of general growth
- Potential impairment of hormonal balance by measuring specific hormones
- Potential impairment of hormonal balance in pups by measurement of foetal testis testosterone levels (very sensitive to anti-androgenic substances)
- Gestation length to confirm if the slight effect seen in the extended 1-generation study were a chance-finding or not.

2.4.2. MoA analysis in non-target organisms other than mammals

A FSTRA was conducted to specifically address the anti-androgenic activity of dimethomorph indicated in mammalian endocrine screening *in vitro* assays (i.e. the YAS assay, see Table 3). The FSTRA with the fathead minnow covers diagnostic endpoints, such as secondary sex characteristics (SSC, i.e. number of tubercles) and vitellogenin (VTG), which are considered suitable to capture anti-androgenic activity in fish (Knacker et al. 2010). In addition, the FSTRA covers an important, non-specific apical endpoint, i.e. reproduction, that may respond to endocrine perturbation and was previously not captured in the available chronic fish tests. Thus, population-relevant endpoints (i.e. growth and reproduction) reflecting the specific protection goal in the chronic risk assessment for aquatic vertebrates (i.e. the population, EFSA AGD 2013) were analyzed in the available chronic fish studies (ELS, FSTRA) with dimethomorph. It should be noted that the androgenised female stickleback screen (AFSS, OECD GD 148) was not considered because it is not fully validated. Hence, it is merely a Guidance Document and there is no mutual data acceptance for this vertebrate test. Additionally, the ecological relevance of the results obtained in the AFSS is questionable when females in the test are manipulated with potent androgen dihydrotestosterone (this is why OECD declined to adopt this test as a test guideline).

In the FSTRA with dimethomorph, no treatment-related effects on apical endpoints (fecundity and growth) nor on diagnostic endpoints (tubercles and VTG) were observed over the 21-d exposure period at or below the MTC (maximum tolerated concentration, i.e. before systemic toxicity occurs; see Wheeler et al. 2013). Hence, the study showed that dimethomorph has no anti-androgenic activity in fish, when tested up to and including the MTC. In conclusion, the anti-androgenic activity of dimethomorph observed in mammalian screening *in vitro* assays was not confirmed in the FSTRA and therefore dimethomorph does meet the ED criteria for the anti-androgenic MoA in non-target organisms other than mammals. In line with the ECHA/EFSA ED GD no additional studies need to be conducted to further investigate the A-modality in non-target organisms other than mammals because this modality is sufficiently addressed for non-target organisms other than mammals with a FSTRA.

The RMS raised some concern about the sensitivity of the FSTRA to detect anti-androgenic activity in fish. However, BASF believes that a FSTRA on fathead minnow is a suitable test system to identify anti-androgenic activity of chemicals as has been demonstrated by the examples of flutamide (Panter et al. 2004, Jensen et al. 2004) and vinclozolin (Martinovic et al. 2008). In general, anti-androgen responses in mammalian test systems (e.g. rat) are more pronounced than those in fish. This may be related to the lower affinity to the androgen receptor in fish than in mammals (Wells and van der Kraak 2000; Panter et al. 2004). Thus, it is not uncommon to detect anti-androgen activity in mammalian screening assays but not in fish. The absence of anti-androgenic activity of dimethomorph in the FSTRA is therefore not necessarily explained by the inability of the test system to detect anti-androgenic activity, but rather by the low activity of dimethomorph in fish.

The RMS's concern was mainly based on the publicly available EPA EDSP data for linuron, which shows one weak positive FSTRA ("guideline study") and one negative (non-guideline) FSTRA from the open literature. However, the deviating results can be explained by the tested concentrations. In the "guideline" FSTRA anti-androgenic effects occurred at 0.92 and 9.1 mg/L, whereas the highest tested

concentration in the second (non-guideline) FSTRA was 0.1 mg/L, which corresponds well with the NOEC of 0.099 mg/L in the "Guideline" FSTRA.

Table 15: Summary of the postulated anti-androgenic mode of action (MoA) analysis for dimethomorph in non-target organisms other than mammals.
VTG = vitellogenin; SSC = secondary sex characteristics; YAS = Yeast Androgen Screen; FSTRA = fish short-term reproduction assay; MTC = maximum tolerated concentration; ELS = early life stage.

Level of Organization	Lines of evidence	Brief description of event	Supporting Evidence
Macromolecular	*In vitro* mechanistic	Antagonism via binding to the androgen receptor	Moderate - evidence in e.g. YAS assay but not in ToxCast data
Cell/Tissue	*In vivo* mechanistic	Decrease in transcription of genes by the androgen receptor	No data
	In vivo mechanistic	Increased VTG levels (e.g. in blood)	No evidence - FSTRA showed no treatment-related effects on VTG
Organ	*In vivo* mechanistic	Feminization of males: reduced SSC in males	No evidence - FSTRA showed no effect on SSC in males at MTC
Individual/Population	Sensitive to, but not diagnostic of A	Impairment of reproductive capacity	No evidence - FSTRA showed no effect on fertility or fecundity
	General adversity	Altered growth rate	Reduced growth rate in fish ELS studies; no effect on adult fish in FSTRA
Conclusion on the postulated MoA: **Dimethomorph has no anti-androgenic potential in non-target organisms other than mammals**, because the observed *in vitro* activity could not be linked to an adverse outcome (i.e. effects on growth).			

Finally, it should be noted that additional OECD TG fish studies would likely add no useful information for the conclusion on the endocrine potential of dimethomorph in fish. If an FSTRA is available, the fish sexual development test (FSDT) and the fish life cycle toxicity test (FLCTT) test have limited added value in detecting endocrine MoA (Dang et al. 2011).

2.5. Conclusion on the ED criteria.

2.5.1. ED criteria for human and mammals

The identification of adversity is based on the definition given in Commission Regulation 2018/605 (and the WHO definition): "*A change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population* ***that result in*** *an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences."*

Slight changes of morphology were observed in rat and dog studies:

- Effects on seminal vesicles are observed only in the extended 1-generation rat study at the high dose without histopathological correlates. No comparable finding was observed in any of the other six rat studies.
- Reduced anogenital distance/index was also observed only at the high dose in the same study. The effect was weak and also associated with slower general development.
- Decreased prostate weight was also seen in the 1-gen study in rats but without histological findings and in dogs with an increase of prostatic interstitial fibrosis only at the high dose of the 90-day study and in all groups in the 12-month study.

These *in vivo* findings could be attributed to an anti-androgenic activity of dimethomorph, confirmed by slight *in vitro* activity at high doses.

Other effects characteristic of anti-androgens such as sperm abnormalities, hormonal changes, malformations of the male sexual tract, nipple retention, histopathological alterations in endocrine organs, were not observed at all in the rat extended 1-generation study with dimethomorph.

In the case of dimethomorph, two fertility studies in mammals are available: one 2-generation study from 1990 and an extended 1-generation study from 2014. In the 2-generation study with doses up to about 70 mg/kg bw/d, no effects on fertility were observed, but as this study was performed according to the old OECD 416 guideline, some parameters for ED assessment were not included. In the extended 1-generation study, slight effects were seen in development at the top dose of about 140 mg/kg bw/d, which can be related to an anti-androgen mode of action. However, no impairment of functional capacities was observed in the first generation. Unfortunately, no second-generation cohort was included in the study, and consequently, **no conclusion can be drawn about potential impairment of functional capacities in a second generation** for the slight effects on development observed in the first generation at the top dose. The effects observed in the first generation may be related to an endocrine activity but are not severe enough to be considered as adverse in the light of the WHO definition and the ED criteria laid down in Commission Regulation (EU) No 2018/60 amending the Plant Protection Regulation No. 1107/2009.

To further address the potential adversity of the slight morphological changes observed in the available studies, a new study is necessary to verify if these changes can lead to functional impairment. Currently, we consider that the effects seen are not adverse enough to consider dimethomorph as an endocrine disruptor. A new "extended" 1-generation study is needed to confirm or disprove the endocrine disrupting properties of dimethomorph.

This new extended 1-generation study would be performed with specific design (4 cohorts) to cover the following end-points:

- Potential functional impairment in the second generation due to the developmental effects observed in the first generation at the top dose
- Potential impairment of pup neurodevelopment
- Potential impairment of hormonal balance by measuring specific hormones

- Potential impairment of hormonal balance in pups by measurement of foetal testis testosterone levels (very sensitive to anti-androgen substances)
- Gestation length

Based on the existing dataset dimethomorph does not fulfil the ED criteria for **wild mammals** as it shows no adverse effects that are considered relevant at the population level, and which are a consequence of an endocrine mode of action. However, a final conclusion on the ED properties in wild mammals needs to consider the findings of the proposed new study. Until then, no additional study is proposed to further address potential ED-related adverse effects of dimethomorph on populations of wild mammals.

2.5.2. ED criteria for non-target organisms other than mammals

Based on the evidence provided in the data package for dimethomorph it can be concluded that **dimethomorph does not meet the ED criteria in non-target organisms other than mammals** in line with Commission Regulation (EU) No 2018/60 amending the Plant Protection Regulation No. 1107/2009 because dimethomorph does not show adverse effects, which could be a consequence of an endocrine mode of action

In the available fish studies (1 FSTRA, 3 ELS tests and one 21-d prolonged toxicity test) dimethomorph showed no endocrine potential, including anti-androgenicity, in fish. Due to the lack of ED-mediated activity in the FSTRA no biologically plausible link between the anti-androgenetic activity indicated in mammalian *in vitro* assays and observed adverse effect(s) in the ELS studies that are relevant for non-target organisms at population level (i.e. effects on growth) could be established. EAS parameters are sufficiently investigated according to the ECHA/EFSA ED GD (2018) and the T-modality is not of concern in case of dimethomorph as shown by the existing mammalian dataset (including regulatory studies and ToxCast/Tox21assays). **Therefore, no further studies in aquatic vertebrates are proposed.**

For birds the available two reproduction studies with dimethomorph provide no evidence for adverse (population-relevant) effects. Accordingly, from the avian studies with dimethomorph there is no indication for endocrine activity or endocrine mediated adverse effects. Based on the existing dataset it appears quite unlikely that dimethomorph fulfills ED criteria in birds. In line with the ECHA/EFSA ED GD (2018) **no further avian studies are proposed.**

References

BASF Documents

Anonymous, 2018, DMM ED GD Full Appendix E, BASF SE, BASF Doc ID 2018/1202685, Unpublished

[redacted] 2018, Summary of Dimethomorph Endocrine Pathway-Related Results in ToxCast/Tox21 Assays, Exponent, BASF Doc ID 2018/1196406, Unpublished

[redacted] 2018, Literature Search report on the endocrine-related literature, Dimethomorph, BASF Scientific Information, BASF Doc ID 2018/1208894, Unpublished

[redacted] 2018, peer Reviewed Scientific Open Literature, BASF SE, Global Ecotoxicology, BASF Doc ID 2018/1193073, Unpublished

Anonymous, 2018, DMM ED GD Appendix E, Ecotoxicology, BASF SE, BASF Doc ID 2018/1227434, Unpublished

Anonymous, 2018, Ecotox Peer Review Literature, BASF SE, BASF Doc ID 2018/1227435, Unpublished

Publications

Dang, Z., Traas, T., Vermeire, T., 2011. Evaluation of the fish short term reproduction assay for detecting endorine disruptors. Chemosphere 85, 1592-1603. **BASF DocID 2011/1300031**

EC (European Commission, Directorate-General for the Environment), Kortenkamp A, Martin O, Faust M, Evans R, McKinlay R, Frances Orton F and Rosivatz E, 2011. State of The Art Assessment of Endocrine Disrupters, Final Report (Project Contract Number 070307/2009/550687/SER/D3). 135 pp. Available online: http://ec.europa.eu/environment/chemicals/endocrine/pdf/sota_edc_final_report.pdf.

ECHA, EFSA, 2018. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/20123 and (EC) 1107/2009. June 2018.

EFSA, 2009. Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438.

EFSA, 2011. Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, EFSA Journal 2011;9(2):2092.

EFSA, 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290.

Filby, A.L., Thorpe, K.L., Maack, G., and Tyler, C.R. 2007. Gene expression profiles revealing the mechanisms of anti-androgen- and estrogen-induced feminization in fish. Aquat. Toxicol. 81(2), 219-231. **BASF DocID 2018/1228158**

Jensen, K.M., Kahl, M.D., Makynen, E.A., Korte, J.J., Leino, R.L., Butterworth, B.C., Ankley, G.T., 2004. Characterization of responses to the antiandrogen flutamide in a short-term reproduction assay with the fathead minnow. Aquat. Toxicol. 70, 99-110. **BASF DocID 2004/1042794**

Knacker, T., Boettcher, M., Frische, T., Rufli, H., Stolzenberg, H.C., Teigeler, M., Zok, S., Braunbeck, T., Schafers, C., 2010. Environmental effect assessment for sexual endocrine-disrupting chemicals, Fish testing strategy. Int. Environ. Assess Manage. 6, 653-662. **BASF DocID 2010/1235172**

Mandibusan M.K and Touart L.W. (2017). A comprehensive review of regulatory test methods for endocrine adverse health effects. Critical Rewiews in Toxicology, 47:6, 440-488. http://dx.doi.org/10.1080/10408444.2016.1272095

Martinovic, D., Blake, L.S., Durhan, E.J., Greene, K.J., Kahl, M.D., Jensen, K.M., Makynen, E.A., Villeneuve, D.L., Ankley, G.T., 2008. Reproductive toxicity of vinclozolin in the

fathead minnow, confirming an anti-androgenic mode of action. Environ. Toxicol. Chem. 27, 478–488. **BASF DocID 2008/1104696**

Marty M.S., et al., 2017. Population-relevant endpoints in the evaluation of endocrine-active substances (EAS) for ecotoxicological hazard and risk assessment. Int. Environ Assess Manage 2017;13(2):317-330. **BASF DocID 2018/1228157**

Panter, G.H., Hutchinson, T.H., Hurd, K.S., Sherren, A., Stanley, R.D., Tyler, C.R., 2004. Successful detection of (anti-)androgenic and aromatase inhibitors in prespawning adult fathead minnows (*Pimephales promelas*) using easily measured endpoints of sexual development. Aquat. Toxicol. 70, 11–21. **BASF DocID 2004/1042797**

Pickford, D.B., 2010. Screening chemicals for thyroid-disrupting activity: a critical comparison of mammalian and amphibian models. Crit. Rev. Toxicol. 1-48. **BASF DocID 2010/1235174**

Wells and Van Der Kraak, 2000. Differential binding of endogenous steroids and chemicals to andogen receptors in rainbow trout and goldfish Environ. Toxicol. Chem., 19, 2059-2065. **BASF DocID 2000/1024618**

Weltje, L., Simpson, P., Gross, M., Crane M., Wheeler, J., 2013. Comparative acute and chronic sensitivity of fish and amphibians: a critical data review. Environ. Toxicol. Chem. 32, 984-994. **BASF DocID 2013/1423720**

Wheeler, J.R., Maynard, S.M., Crane, M., 2014. An evaluation of fish early life stage tests for predicting reproductive and longer-term toxicity from plant protection product active substances. Environ. Toxicol. Chem. 8, 1874-1878. **BASF DocID 2014/1332572**

Wheeler, J.R., Panter, G.H., Weltje, L., Thorpe, K.L., 2013. Test concentration setting for fish in vivo endocrine screening assays. Chemosphere 92:1067-1076. **BASF DocID 2013/1423722**