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Justification of minority position in classification of dimethomorph for reproductive toxicity

as Repr. 2; H361fd

prepared by two RAC members: Bogusław Barański and Ralf Stahlmann

During a plenary session on 20 September 2019 RAC adopted the opinion on harmonised classification of dimethomorph as Repr. 1B; H360F May damage fertility, while two RAC members, listed above, announced minority position preferring classification of dimethomorph for reproductive toxicity Repr. 2fd.

In the draft RAC opinion (27/08/2019) and during discussion at plenary session the main effects justifying the classification Repr. 1B; H360F were: the reduction in gestation duration, decreased AGD in male and female pups, delayed puberty onset in combination with alterations in weight of testes and sex accessory organs in exposed animals.

Gestation length

The classification is partly based on findings of shortening of gestation length in the 2-generation reproductive toxicity study (2-Gen) and in extended one generation reproductive toxicity study (EOGRTS) as shown in table below

Table: Summary of gestational length in the 2 generation and extended one generation study in rats

	Gestation duration (days)					
Dose (ppm)	**0**	**100**	**300**	**800**	**1000**	**1600**
2-Gen P0	22.0 ± 0.3	22.1 ± 0.3	21.9 ± 0.4		**21.8 ± 0.4***#	
2-Gen F1A	21.9 ± 0.4	21.9 ± 0.3	22.1 ± 0.3		**21.7 ± 0.5***#	
2-Gen F2B	21.9 ± 0.2	22.2 ± 0.5	21.9 ± 0.3		21.8 ± 0.4	
EOGRTS	22.3		22.2	22.0		**21.4****
HCD	21.5 - 22.3					

*p<0.05 (ANOVA), **p<0.01 (Dunnett test); # upon re-evaluation using Dunnett test, statistical significance was not reached

In our view shortening of gestational length in 2-generation studies observed only at the top dose is due to natural variation of this parameter in female rats (difference is equal or less than one standard deviation of any group) , and is not statistically significant in comparison between control and top dose group).

In EOGRTS reduction of gestation duration was only observed at the top dose from 22.3 to 21.4 days (no standard deviation was provided, thus there is no information on variation of this parameter within groups), and can also be associated with slight maternal toxicity (see table below).

Table: Body weight development of F0 females during pre-mating and gestation phases in EOGRTS

Dose level [ppm]	**0**	**300**	**800**	**1600**
Body weight (GD 20)		↓ (3%)	↓ (1%)	↓ **(6%)***
Body weight gain (GD 0-20)		↓ (7%)	↓ (3%)	↓ **(11%)***
Body weight (PND 21)		↓ (1%)	↓ (1%)	↓ **(4%)***
Food intake (PND 1-21)		↓ (2%)		↓ **(6%)***

Similar body weight decrease only at the top dose was seen in 2-generation reproductive toxicity study, so both top doses in 2-generation and in EOGRTS were similarly toxic to dams

Table: Mean body weight and mean body weight gain in females of P0 and F1 generations in 2 generation study

Dose level (ppm)		0	100	300	1000
P0	BW, week 15 (g)	292.3	291.2	280.8	**267.2*↓ (8,6%)***
	BW gain, week 1-15 (g)	138.0	137.5	128.3	**117.7* ↓ (14.7%)***
	Food consumption week 1-14 (g)	18.3	18.5	18.3	17.5
F1	BW, week 15 (g)	292.4	285.2	281.0	277.4
	BW gain, week 1-15 (g)	183.4	178.2	174.4	171.0

* = significantly different p < 0.5; analysis of variance with one factor treatment followed by Student Newman-Keuls test

So in our opinion the slight effect alone on duration of gestation in dams showing slight systemic toxicity , still being within HCD of range of 21.5 - 22.3 days, is not sufficiently adverse to justify its classification neither to category 1 or 2, although it might be considered as supportive evidence for classification to category 2 of reproductive toxicity.

Decreased AGD in male and female pups

As provided by Dossier Submitter in CLH report the analysis of anogenital (AG) distance in male and female pups based on litter data showed that only for males the changes at the top dose are statistically significant and slightly outside the historical controls. For females, the analysis based on litter data confirmed that no treatment-related effects are noticed. These data alone are not sufficient for classification, but may be used as supportive data on developmental toxicity .

Effects on male reproductive system

There is either no data on spermatogenesis or number or quality of sperm in 2-generation study or no evidence in EOGRTS on any effect of dimethomorph spermatogenesis or number or quality of sperm. No data on alteration of estrous cycle. In EOGRTS reduced weight of prostate at all doses, seminal vesicles at mid and top doses, and increased weight of testes at top dose in F1A/F1B cohorts (??), but no data were provided for F0 generation of parental males. Decreased weight of prostate and increased testes weight was also observed in 90-day repeated -dose toxicity study and 52-week carcinogenicity study in dogs.

These data om alteration of weight of testes and accessory sex organ do not provide sufficient evidence for classification but may be used as supportive evidence for effect on reproductive organs.

Puberty onset

No data on puberty onset and on AGD in male and female pups were provided for 2-generation reproductive toxicity study, since they were not determined in this study. Therefore the assessment of effect of dimethomorph on onset of puberty is solely based on results of second study – EOGRTS>

In EOGRTS the puberty onset in male and female pups were affected.

Females. The age of vaginal opening was delayed by 2 days and this delay was statistically significant at 1600 ppm ($p<0.01$), exceeding the range of the submitted historical control data (Table below). According to the authors of the study, this delay by 6% in comparison with control group was due to a decrease in body weight (i.e., slowed general development) at this dose level: body weight on PND 21 was significantly reduced by 8% while there was no significant difference in the body weight on the day of vaginal opening. It is noted that neither in CLH report nor in draft RAC opinion data on body weight or body weight gains of female and male pups were provided for the period from the end of

lactation (21 day post-partum) till vaginal opening or preputial separation. Delayed growth of pups during lactation showing systemic toxicity in pups of dams exposed to 21 days post-partum could delay the onset of puberty, thus it could be at least partial secondary unspecific effect.

Table: Sexual maturation of F1 pups: age of vaginal opening in females

parameter	**Age of vaginal opening**			
Dose (ppm)	**0**	**300/150**	**800/400**	**1600/800**
Pups examined	40	40	40	40
Days to criterion	31.4	31.9	32.0	**33.4****
Historical control range (d)	30.0 - 32.1			
Additional historical control data (d)	29.5 - 31.9#			
Body weight at criterion (g)	96.4	96.9	95.9	96.9
Historical control range (g)	86.4 - 99.6			
Additional historical control data (g)	83.1 - 100.7#			

** $p \leq 0.01$ (Dunnett-test, two-sided); # additional historical control data (2010-2015) for vaginal opening as provided by the applicant upon request by EFSA during the renewal application

Males. The preputial separation was statistically significant delayed at both 800 ppm and 1600 ppm dose levels (43.7 and 47.9 days versus 42.0 days in controls). Compared to controls, body weight of F1 males on PND 21 was slightly (3%) and statistically significant (9%) reduced in the mid and high dose groups, respectively. However, after cessation of exposure, an overall increase of body weight was faster in pups of 800ppm and 1600 ppm than in control group being 2.2% and 12.9% higher than body weight at preputial separation in control group.

The delay in preputial separation in mid dose males (800 ppm) was considered minimal (age at preputial separation was 4% higher than control value) and related to overall growth retardation, particularly before weaning. For the high dose group 1600 ppm an age at preputial separation was 14% bigger than in control group, thus specific effect on preputial separation cannot be excluded.

Table: Sexual maturation of F1 pups: age of preputial separation in males

parameter	**Age of preputial separation**			
Dose (ppm)	**0**	**300/150**	**800/400**	**1600/800**
pups examined	38	40	39	39
Days to criterion	42.0	41.8	**43.7****	**47.9****
Historical control range (d)	39.7 - 42.5			
Additional historical control data (d)	40.5 - 45.2 ##			
Body weight at criterion (g)	176.6	174.0	180.5	**199.5****
Historical control range (g)	156.5 - 181.0			
Additional historical control data (g)	168.1 - 195.3 ##			

** $p \leq 0.01$ (Dunnett-test, two-sided); ## additional historical control data (2010-2015) for preputial separation as provided by the applicant upon request by EFSA during the renewal application

In our opinion the delayed puberty in both sexes was observed only in the groups exposed at top dose. This delayed puberty onset is not fully explained by reduced body weight of animals, nevertheless body weight of these animals was significantly reduced at day of birth until weaning, and it is not known how the body weight of these animals was changing between 21 day post-partum till puberty. The exposure of offspring was most probably stopped after weaning in line with standard procedure in EOGRTS , but It is not known when exposure was ended since the robust study summary for this study was not provided.

We also note that in EOGRTS dimethomorph at any dose did not affect any other indexes related to fertility, although at the highest dose a decreased food consumption, reduced body weight/body weight gain and changes in clinical chemistry and pathological changes in liver were observed.

In the both reproductive toxicity studies no effect on the mating index, fertility index, gestation index, live birth index and sperm parameters was observed, although slight parental toxicity was observed in both studies. Presentation of these negative findings in CLH report would facilitate analysis of data, but the conclusions presented by Dossier Submitter are trusted.

It would be convenient for evaluation of data to have an access on CIRCABC to RAR for dimethomorph, particularly its part related to assessment of health effects, which was prepared by Dossier Submitter. As informed in CLH report Dimethomorph is part of the AIR3 renewal programme for active substances (Commission Implementing Regulation (EU) No 844/2012). For a renewal-application under Regulation (EC) 1107/2009, the compound is currently being re-evaluated with member state The Netherlands as rapporteur member state (RMS). The RAR was peer reviewed by the Co-Rapporteur Member State Germany. This process is currently ongoing. A draft assessment report (including a proposed decision) of the Netherlands has been published by EFSA for public consultation April 10th 2018.

Comparison with classification criteria

The adverse effect on onset of puberty is included within the adverse effects on sexual function and fertility (Annex I of CLP Regulation point: 3.7.1.3.) but in this case taking into account reduced body weight of these animals at birth lasting until weaning it cannot be excluded that effect is also related to adverse effect induced during pregnancy or a result of maternal exposure thus fulfilling the requirement given in point 3.7.1.4. :" Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation." Therefore in our opinion, the adverse effect on puberty onset can be considered for classification of both: fertility effects and developmental toxicity

The adverse effect on puberty alone (delay of puberty onset by 6% in females and 14% in males) only at the top dose exerting maternal toxicity, reducing body weight of pups at birth and during lactation does not provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects meeting criteria for category 1B. Based on available data it is not possible to exclude that this effect, occurring together with other toxic effects, is not partly a secondary non-specific consequence of other toxic effects. In addition as admitted by Dossier Submitter no effect on the mating index, fertility index, gestation index, live birth index and sperm parameters was observed even at the top dose. Therefore in our opinion dimethomorph warrants classification as Repr. 2 H361fd , because there is some evidence (delayed onset of puberty) from experimental animals, supplemented with other information on slightly decreased gestational length, decreased AGD in male pups and effects on weight of testes, seminal vesicles and prostate. The evidence is not sufficiently convincing to place the substance in Category 1. It is also noted that deficiencies in the studies make the quality of evidence less convincing, therefore Category 2 could be the more appropriate classification.