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Comment by the DE-CA on the CLH proposal for dimethomorph as Repr. 1B

Reproductive toxicity:

With regard to the proposed classification, effects on sexual function and fertility and those on development should be considered separately.

a) Adverse effects on sexual function and fertility

For dimethomorph classification as Repr. 1B for effects on sexual function and fertility is proposed. This proposal is based on a statistically significant reduction in gestation length (21.4 days vs. 22.3 days in control) in the high dose group (1600 ppm/144 mg/kg bw/d) of the extended one generation study (2014a) in Wistar rats. The concurrent control was at the upper end of the historical control range (21.5 – 22.3 days). Since the average gestation length in the high dose group was only slightly below the lower range of the historical control data, we would appreciate if the dossier submitter could deliver information on the respective individual animal data. Knowledge on the distribution of individual gestation lengths might further help in the evaluation of this parameter (e.g. with regard to the presence of outliers).

The reduced gestation length was observed in the presence of maternal toxicity. The DS argues that the reduced gestation length is unlikely to be secondary to the reduction in food consumption, body weight and body weight gain. We agree with this evaluation.

Furthermore, from the perspective of the DS the evidence for a reduced gestation length in the EOGRTS is supported by a slight, though not significantly, reduced gestation length in the 2-generation study (1990). In fact, there was a slight but significant (ANOVA) decrease in gestation length in the parental generation (21.8 days at 1000 ppm as compared to 22.0 day in the control group) and in the F1 generation to produce the F2A litters (21.7 vs. 21.9 days). Re-evaluation by the applicant using the two-sided Dunnett test did not reveal statistical significance. Thus, the effect on this parameter in the previous study is equivocal. In conclusion classification as Repr. 1B (H360F) based on a reduced gestation length only is questionable; Instead, this finding alone might be rather suggest category 2.

From our point of view, in contrast, a classification for effects on sexual function and fertility as Repr. 1B (H360F) should rather be based on effects in male animals. A delay in puberty (delay in preputial separation) was observed in the high dose group (1600 ppm/144 mg/kg bw/d) of the extended one generation study (2014a) in Wistar rats. According to CLP Regulation (Annex I, 3.7.1.3), adverse effects on onset of puberty may result in classification for effects on sexual function and fertility. The delayed sexual maturation and the observed reduced anogenital distance (discussed below) appear to reflect the anti-androgenic effects of dimethomorph observed in in vitro

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assays (YAS assay, MDA-kB2 reporter gene assay). Furthermore reduced seminal vesicle and prostate weight were observed in rearing males in the EOGRTS.

A reduction in prostate weight and an increased incidence of prostatic interstitial fibrosis were observed at 1350 ppm (approx. 43-47 mg/kg bw/d) in a 90-day and in a 1-year repeated dose studies (1986 and 1990) in dogs.

We rate the relevance of the prostate findings in dogs and rats much higher than the DS and consider them a second line of evidence to support the proposal. It was noted that both the 90-day and the one-year study in Beagle dogs have been assigned reliability 1 by the DS in Table 14 but the relevant findings are only briefly mentioned. Therefore, in the following, some additional information is given.

In the 90-day feeding study, a very strong decrease (not an increase as erroneously stated in the CLH Report on page 16) in absolute (-62%) and relative prostate weight was noted at the top dose level of 43 mg/kg bw/day, accompanied by increased interstitial fibrosis in all high dose males. The reduction in prostate weight and the histopathological findings were reproducible in the one-year study at nearly the same dose (47 mg/kg bw/day). Mean absolute organ weight was diminished by 48%, and the degree of interstitial fibrosis in all high dose males was increased. The following information has been copied from the most recent version of the RAR:

Table 1: 90-day dietary toxicity study in dogs – prostate weight

Dose (mg/kg bw/day)	**Control**	**5**	**15**	**43**
Absolute organ weights, group mean value (g) ± SD				
Prostate	8.56 ± 3.67	6.63 ± 1.18	6.49 ±2.08	**3.27 ± 1.31****
Relative organ weights (as % body weight), group mean value ± SD				
Prostate	0.070 ± 0.026	0.060 ± 0.008	0.057 ± 0.023	**0.027 ± 0.010****

*** = significantly different from control, p < 0.01 ("F-max" test; parametric ANOVA; Student's t-test)*

Table 2: 90-day dietary toxicity study in dogs – histopathological prostate lesions

Group/dose (ppm)	**Control**	**150**	**450**	**1350**
Dose (mg/kg bw/day)	**Control**	**5**	**15**	**43**
Number of animals	**4**	**4**	**4**	**4**
Prostatitis		1		2
Increased fibrosis				**4***

** = significance of differences in a pairwise (Fisher`s) test between each treatment and control incidence: p < 0.05*

Table 3: One-year dietary toxicity study in dogs – prostate weight

Group/dose (ppm)	**Control**	**150**	**450**	**1350**
Dose (mg/kg bw/day)	**Control**	**4.9**	**14.7**	**44.6**
Prostate – absolute	8.25	8.97 (+9%)	5.55 (-33%)	**4.27*** (-48%)
Prostate – adjusted	7.40	9.04 (+22%)	6.23 (-16%)	4.36 (-41%)**

**= significantly different from control, p < 0.05 (analysis of covariance); values adjusted using body weight of covariate*

Table 4: One-year dietary toxicity study in dogs – histopathological prostate lesions

Group/dose (ppm)	**Control**	**150**	**450**	**1350**
Dose (mg/kg bw/day)	**Control**	**5.0**	**15.7**	**47.0**
Number of animals	4	4	4	4
Minimal interstitial fibrosis	2	2	2	1
Mild interstitial fibrosis	2	1	1	0
Moderate interstitial fibrosis	0	1	1	3

It is quite obvious that the prostate is a target organ of dimethomorph in the dog. A threshold for both effects seems to exist because, in the two studies, the next lower dietary dose of 450 ppm was the NOEL.

An "increase of interstitial fibrosis" means that the ratio between glandular and connective tissue is skewed towards the latter. In textbooks of anatomy, it is described that the ratio of the two tissue types in the dog differs with age. The quantitative share of connective tissue (as compared to the glandular) is higher in young dogs and will diminish when the animals get older. (In really old dogs, however, the ratio can be extremely variable but this is not relevant for the studies under evaluation.) Because of this knowledge, one might speculate that the normal morphological development of the prostate was somehow delayed by high doses of the test substance. Such an assumption could also provide an explanation why the effects do not become more pronounced with longer administration but were similar or even more severe after 90 days of feeding.

Occurring in parallel, lower organ weight and higher proportion of connective tissue results in a reduction of functionally active glandular tissue and, thus, may have an impact on the production of prostate secrete which is needed to ensure sperm motility. Therefore, an impact on male fertility may be reasonably expected even though no data on reproduction in dogs is available. It must be emphasized that, with regard to effects on the prostate and their potential impact on reproduction, the dog is certainly a better model for man than the rat in which even surgical removal of this organ will not necessarily mean infertility. Accordingly, the prostate effects in dogs should be considered adverse to male fertility and clearly support classification for reproductive toxicity.

We consider the effects on the prostate in the dog studies to be well in line with anti-androgenic properties. Indeed, prostate changes are common in ageing dogs due to physiological hormonal changes but the dogs even in the one-year study are still rather young, compared to life expectancy of the species. They cannot be considered "ageing" as it is the case, e.g., in rats in the second year of a long-term study. There are further arguments to consider the lower prostate weight and the histological changes endocrine-mediated: "Development and maintenance of the prostate gland (…) are dependent on androgen stimulation. In the adult, DHT, formed from testosterone by 5-alpha-reductase activity within epithelial cells of the active tissue, is the effector androgen." [Cited from: Vidal, J.D. et al. "Reproductive System and Mammary Gland" in: "Toxicologic Pathology. Nonclinical Safety Assessment" (eds. Sahota, Popp, Hardisty and Gopinath), CRC Press, Boca Raton (FL/USA), 2013, ISBN978-1-4398-7210-9, page 751.] The pharmacological substance finasteride which is antiandrogenic because of inhibition just of this enzyme is widely used to control benign prostate hyperplasia in man. It is approved for the same indication in dogs. Its desired effect is a reduction of prostate size. The same was seen in dogs when treated with high doses of dimethomorph.

In addition, in a 104-week chronic toxicity study (1990) and in a 2-year carcinogenicity study (1991) in SD rats an increased incidence of benign testicular lesions (i.e., of focal hyperplasia and adenoma) was observed at 2000 ppm (approx. 97 mg/kg bw/d). An increase in testes weight was already detected in rearing males in the EOGRTS.

On balance, all these effects on male reproductive organs in rats and dogs may be considered as indicative for possible effects on male fertility.

b) Adverse effects on development of the offspring

The proposed classification Repr. 1B for developmental toxicity for dimethomorph is based on reduced anogenital distance, delayed puberty, reduced pup weight and reduced seminal vesicle and prostate weight in the EOGRTS (2014a) in Wistar rats. We question if these effects clearly support a classification as Repr 1B for developmental toxicity. Post-natal exposure of the pups might have had an influence on the latter effects. Hence, due to the study design one may not clearly distinguish whether intrauterine or post-natal exposure had caused the effects. As mentioned above according to CLP Guidance (2017), adverse effects on onset of puberty and changes of reproductive organs usually result in classification for effects on sexual function and fertility. In our view, the slight reduced pup weight (up to 12%) would not warrant a classification.