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Position Paper on the proposed classification of Dimethomorph

(BAS 550 F)

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Executive Summary

In the draft Risk Assessment Report received from the Rapporteur Member State, a classification as Repr. Cat 1B (H360 FD) is proposed for Dimethomorph.

According to the RMS, this classification is supported by the effects seen in the extended 1-generation study:

- on anogenital distance which is considered to be a developmental effect
- and on preputial separation which is regarded as an effect on sexual function and fertility.

The notifier strongly disagrees with this proposal and considers that the effects induced by dimethomorph are not severe enough to fulfil the criteria for category 1B.

On a weight of evidence basis, these effects seen in isolation without other effects on fertility and developmental parameters are considered to be of transient nature and of low severity and do not trigger classification for reprotoxicity in Cat. 1B.

This is underlined by a review performed on recent ECHA decisions, looking for substances classified in Cat. 1B that were mentioned to have effects on anogenital distance or sexual maturation. For these substances the effects which trigger the classification according to the opinion of the RAC have been listed.

According to ECHA decisions, no substances were classified in Cat 1B for effects on anogenital distance or preputial separation only.

For classification in Cat 1B for fertility, the main drivers for classification were:

- Decreased fertility
- Decreased sperm count
- Decreased litter size
- Decrease in fertility index
- Decrease in mating index
- Severe effects on sexual organs including histopathological changes

For classification in Cat. 1B for development, the main drivers for classification were:

- Stillbirth
- Post-natal mortality
- Malformations
- Effects on sexual maturation associated with malformations of the male reproductive tract.

These severe effects are not observed with dimethomorph, therefore it is considered that the RMS proposal to classify dimethomorph in Cat 1B for both fertility and development is clearly disproportionate and that this proposal should be reconsidered.

Background

In the draft Risk Assessment Report, the Rapporteur Member State in charge of the assessment of dimethomorph has proposed classification for reproductive toxicity as reprotoxic category 1B for both fertility and development (H360 FD) based on the following reasoning:

"Based on the information a non-maternally mediated mode of action through the anti-androgenic properties of dimethomorph seems likely. The observed effects are therefore considered to be related to the anti-androgenic effect of dimethomorph and not secondary to maternal toxicity. This mode of action is considered to be relevant to humans and therefore classification for developmental toxicity is necessary. The effect on anogenital distance is considered to be a developmental effect while the effect on preputial separation can be regarded as an effect on sexual function and fertility as Regulation (EC) No 1272/2008 states that "*This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty…*".

Based on the observed **decrease in anogenital distance** and **delay in preputial separation**, taking into they are not related to maternal toxicity and are considered as relevant to humans, it is proposed to classify dimethomorph as reproductive toxicant category 1B (H360FD)."

In this position paper, the notifier will demonstrate that this classification proposal is not appropriate. A summary of effects observed in studies performed with dimethomorph is provided and a comparison with other harmonised ECHA classifications has been performed to conclude that, on a weight of evidence basis, this proposal is clearly disproportionate.

I - Data on Dimethomorph

Dimethomorph is currently not classified for toxicity according to the Regulation (EC) No 1272/2008. A 2-generation study was available to support this non-classification ([redacted], 1990, Doc ID DK-430-001) For the purpose of the renewal an extended 1-generation was performed ([redacted] 2014a, Doc ID 2014/1181670) to cover the most recent endpoints cited in the revised guideline OECD 416 which were not measured in the previous study:

- Sperm parameters.
- Oestrous cycle length.
- Age of vaginal opening, preputial separation and anogenital distance.
- Organ weights.

The observed effects are summarized below.

I.1 Maternal Toxicity

Maternal toxicity was observed at the high dose only, consisting of:

- decrease of food consumption,
- decrease of body weight and decrease of body weight gain (see table 1-3).

Hepatotoxicity was also observed at the high dose, consisting mainly of:

- increase of liver weight,
- hypertrophy accompanied with histopathological effects (lymphoid infiltration (see table 5).

Table 1 – Maternal body weight during gestation and lactation

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Body weight [g]								
Gestation phase	N = 24		N = 24		N = 25		N = 22	
GD 0 Δ% (compared to control)	223.8	13.5	223.0 -0.4	15.7	223.5 -0.1	13.6	216.5 -3.3	13.0
GD 7 Δ% (compared to control)	245.9	16.0	240.5 -2.2	16.6	244.6 -0.5	13.5	235.6 -4.2	12.0
GD 14 Δ% (compared to control)	269.6	18.1	262.6 -2.6	18.1	267.6 -0.8	13.9	**256.4*** -4.9	13.0
GD 20 Δ% (compared to control)	332.6	23.7	323.8 -2.7	27.7	328.7 -1.2	19.9	**313.7*** -5.7	19.0
Lactation phase	N = 24		N = 23		N = 25		N = 22	
LD 1 Δ% (compared to control)	252.8	17.0	247.2 -2.2	16.4	250.5 -0.9	13.1	245.8 -2.8	16.9
LD 4 Δ% (compared to control)	260.7	19.0	259.5 -0.5	20.0	265.4 1.8	13.8	256.0 -1.8	15.5
LD 7 Δ% (compared to control)	262.7	16.1	264.3 0.6	11.2	265.1 0.9	13.0	257.0 -2.2	9.2
LD 14 Δ% (compared to control)	289.9	19.1	282.8 -2.4	19.1	288.6 -0.5	12.1	**276.1*** -4.8	14.2
LD 21 Δ% (compared to control)	279.7	14.4	278.2 -0.5	18.4	277.6 -0.7	10.5	**269.0*** -3.8	15.0

* = p<0.05; Dunnett test (two-sided)
GD = gestation day; LD = lactation day

Table 2 – Maternal Body weight gains during gestation

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Body weight gain [g]								
GD 0-7 Δ%	22.2	5.3	**17.9*** -20.8	4.3	21.1 -4.5	5.9	19.2 -13.6	4.1
GD 7-14 Δ%	23.7	3.6	21.5 -6.8	4.2	23.0 -3.0	4.5	**20.8*** -12.2	3.3
GD14-20 Δ%	62.9	12.8	61.2 -2.9	14.2	61.2 -3.0	10.5	57.3 -9.0	9.7
GD 0-20 Δ%	108.8	15.3	100.8 -7.4	18.0	105.2 -3.3	13.6	**97.2*** -10.7	9.9

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

Table 3 – Food consumption of parental females during gestation and lactation

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Food consumption [g/rat/day]								
Gestation phase	N = 24		N = 24		N = 25		N = 22	
GD 1-7	18.4	1.8	17.7	1.8	18.7	1.1	17.7	1.7
Δ% (compared to control)			-3.4		1.8		-3.8	
GD 7-14	20.7	1.9	19.9	2.0	20.4	31.3	19.7	1.9
Δ% (compared to control)			-4.0		-1.3		-4.8	
GD 14-20	21.0	1.2	22.1	1.7	21.9	1.0	21.6	1.6
Δ% (compared to control)			5.1		4.6		2.8	
LD 0 -20	181.0	129.2	211.9	102.6	191.3	120.3	184.0	117.1
Δ% (compared to control)			-17.1		5.7		1.6	

* = p<0.05; Dunnett test (two-sided)
GD = gestation day

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Food consumption [g/rat/day]								
Lactation phase	N = 24		N = 23		N = 25		N = 22	
LD 1-4	32.0	4.0	32.2	4.8	33.9	4.4	31.2	5.3
Δ% (compared to control)			0.6		6.0		-2.4	
LD 4-7	38.4	2.9	39.2	3.1	39.7	3.2	37.0	2.7
Δ% (compared to control)			2.1		3.3		-3.7	
LD 7-14	54.1	4.5	53.1	5.2	54.3	3.4	**50.7***	5.6
Δ% (compared to control)			-2.0		0.2		-6.3	
LD 14-21	66.4	4.8	63.5	10.1	64.8	3.1	**61.3***	5.3
Δ% (compared to control)			-4.3		-2.3		-7.6	
LD 1-21	52.7	3.6	51.5	5.3	52.7	2.8	**49.4***	4.5
Δ% (compared to control)			-2.3		0.0		-6.2	

* = p<0.05; Dunnett test (two-sided)
LD = lactation day

Table 4: Summary of female reproduction and delivery data.

Parental generation	**F_0 females**			
Dose [ppm]	**0**	**300 / 150**	**800 / 400**	**1600 / 800**
Animals per dose [n]	25	25	25	25
Female fertility				
- placed with males [n]	24	25	25	25
- mated [n]	24	25	25	25
- mating index [%]	100	100	100	100
- pregnant [n]	24	24	25	22
- **Fertility index** [%]	100	96	100	88
HCD for fertility index: 84-100 %				
Mating days until Day 0 pc	2.2	2.7	2.2	2.4
Duration of gestation[days]	22.3	22.2	22.0	**21.4****
HDC for Gestation days: 21.5 – 22.3				
Implantation sites, total[n]	290	276	305	258
- per dam [n]	12.1	11.5	12.2	11.7
Post implantation loss[n]	14	22	11	15
- per dam [n]	0.6	0.9	0.4	0.7
- per litter [mean %]	4.7	8.0	3.6	5.7
Females with live-born	24	23	25	22
- **Gestation index** [%]	100	95.8	100	100
- with stillborn pups [n]	2	0	0	0
- with all stillborn [n]	0	0	0	0
Pups delivered [n]	273	263	293	245
- per dam [mean n]	11.4	11.4	11.7	11.1
- live-born [n]	269	263	293	245
- stillborn [n]	4	0	0	0
- **Live birth index** [%]	98.5	100	100	100

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

Table 5 – Histopathological effects on the female liver F1

Liver	**Female animals**			
Test group [ppm]	**0**	**300**	**800**	**1600**
Organs examined	24	25	25	25
Hypertrophy, centrilobular	0	0	0	13
• Grade 1				11
• Grade 2				2
Apoptosis, increased	0	0	0	5
• Grade 1				5
Infiltration, lymphoid	24	25	25	25
• Grade 1	23	18	21	12
• Grade 2	1	7	4	7
• Grade 3				6

For the extended 1-generation study it can be concluded that a moderate maternal toxicity was seen at the high dose only.

In the 2-generation study, maternal toxicity was also seen at the highest tested dose (1000 ppm) consisting of reduction in pre-mating body weight gains. These effects

were less pronounced than those seen in the extended 1-generation and were limited to the pre-mating period.

Table 6 – Body weight and body weight gain in the 2-generation study during pre-mating period

Dose level (ppm)		M				F			
		0	**100**	**300**	**1000**	**0**	**100**	**300**	**1000**
F0	**Bodyweight** (g)								
	Week 5	362.5	360.7	356.2	368.2	222.5	221.0	217.0	**206.8***
	Week 15	526.5	525.8	511.0	543.0	292.3	291.2	280.8	**267.2***
	Weight gain (g)								
	Week 1-15	332.6	334.3	320.2	352.2	138.0	137.5	128.3	**117.7***
	Food consumption (g)								
	Week 1	24.9	24.5	24.6	24.5	18.1	17.8	18.1	**16.3***
	Week 2	26.7	25.8	25.6	26.2	18.2	18.2	17.9	**17.0***
	Week 3	27.4	26.9	26.8	27.3	19.0	19.1	18.9	**17.7***
	Week 4	28.6	27.4	27.5	28.0	19.4	19.5	19.2	**18.0***
	Week 5	28.2	27.1	26.8	27.8	19.0	19.2	18.7	**17.3***
	Week 1-14	27.4	26.3	26.0	27.0	18.3	18.5	18.3	17.5
F1	**Bodyweight** (g)								
	Week 15	521.6	520.0	507.2	520.2	292.4	285.2	281.0	277.4
	Weight gain (g)								
	Week 1-15	397.8	399.6	385.0	398.6	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

I. 2 - Effects on development

In the extended 1-generation study, a decrease of the F1 pup weight was seen at the high dose only on each day of measurement. It should be mentioned that no effects were seen on pup survival (see table 7). No effects on pup survival and pup weight were observed in the 2-generation study, for each generation at the highest tested dose (1000 ppm) despite a slight maternal toxicity (see table 6).

Table 7– F1 pup survival and pup weight.

Pup generation	**F_1**			
Dose [ppm]	**0**	**300 / 150**	**800 / 400**	**1600 / 800**
Number of litters	24	23	25	22
- with liveborn pups	24	23	25	22
- with stillborn pups	2	0	0	0
Pups live-born [n]	269	263	293	245
Pups stillborn [a] [n]	4	0	0	0
Pups died [n]	0	2	2	1
Pups cannibalized [n]	0	1	3	7
Pups culled day 4 [n]	77	76	91	67
Pups day 4 - pre-cull [n]	269	260	289	239
Viability index [%]	100	99.1	98.7	97.6
Pups day 4 - post cull [n]	192	184	198	172
Pups day 21 [n]	191	184	197	170
Lactation index [%]	99.5	100	99.6	98.9
Sex ratio [% live males]				
Day 0	53.8	50.5	50.5	46.1
Day 21	51.8	51.1	51.1	47.7
Male pup weight [g]				
PND 1 [g]	7.0	7.0	6.7	**6.2****
PND 4 [g]	10.8	10.3	10.3	**9.5****
PND 7 [g]	16.9	16.3	16.5	**15.3****
PND 14 [g]	34.0	32.8	33.7	**31.9****
PND 21 [g]	54.6	52.6	53.1	**49.5****
Male body weight gain				
PND 1 to 21 [g]	47.6	45.7	46.3	**43.3****
Female pup weight [g]				
PND 1 [g]	6.6	6.6	6.4	**5.8****
PND 4 [g]	10.2	10.0	10.0	**9.0****
PND 7 [g]	16.0	15.7	16.0	**14.6****
PND 14 [g]	32.7	32.0	32.9	30.9
PND 21 [g]	52.2	50.8	51.4	**47.8****
Female body weight gain				
PND 1 to 21 [g]	45.5	44.2	45.0	**42.0****

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

Therefore, this effect on pup body weight seen in the extended 1-generation is considered to be secondary to maternal toxicity.

The only other effect observed for developmental landmarks is a slight decrease of anogenital distance (AGD) and anogenital index (AGI) in males of the high dose group (see table 8). It has to be noted that the AGD of the control group is outside the concurrent Historical Control Data (HCD) whereas the AGI of the control group is just at the higher end of HCD. AGD and AGI are clearly in the HCD for the low dose group (300 ppm). The AGI of the mid dose (800 ppm) is within the historical control whereas the AGI of the high dose group (1600 ppm) is borderline below the HCD (1.57 vs 1.58). No strong relation to dosing is observed for the AGI.

Overall, it can be considered that this effect is very weak.

Table 8 – Effects on AGD and AGI in males in the extended 1-generation.

Dose level [ppm]	**0**		**300**		**800**		**1600**	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
PND 1								
male pup	N = 146		N = 129		N = 145		N = 112	
AG distance [mm]	3.15	0.19	**3.09***	0.23	**2.95****	0.19	**2.85****	0.25
Historical Control [mm]	2.99–3.15 **(3.13 if current study data excluded)[1]**							
AG index	1.65	0.09	**1.62***	0.11	**1.58****	0.08	**1.57****	0.12
Historical Control	1.58–1.67							

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

1 – There is a mistake in the report, generally values reported in a study should not be included in the Historical Control Data. The correct values of HCD are 2.99 – 3.13. This has to be corrected also in the RAR.

The anti-androgenic mode of action (MoA) of dimethomorph could explain such an effect, but it has to be noted that no effects on other sensitive developmental landmarks as nipple retention (see table 9) were seen and that no malformations were observed in the male genital tract. Therefore, even though the decreased AGD might be explained by this MoA, it has to be concluded that it is **a very slight effect** with reduced pup weight as a confounding factor.

Table 9 – Effects on nipple development in males

Dose level [ppm]	**0**		**300**		**800**		**1600**	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Nipple development [%]								
Lactation male pup	N = 24		N = 23		N = 25		N = 22	
Day 12	95	10	95	13	95	13	99	4
Day 20	0	0	0	0	0	0	0	0

I.3 - Effects on fertility

According to the RMS, effects seen in the extended 1-generation on preputial separation are considered to warrant classification, and in addition, effects on gestation lengths should also be taken into account.

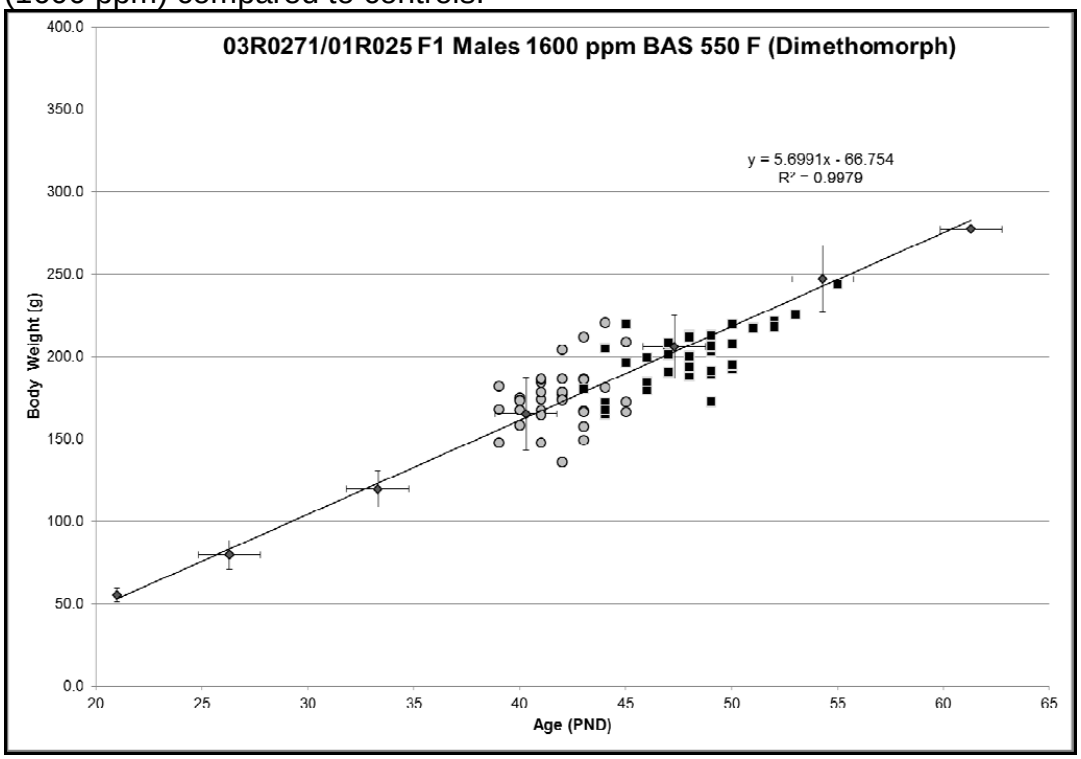
For preputial separation, slight effects are seen only in the high dose treated males (see table 10 and figure 1). When considering the individual data for the time of preputial separation, there is no trend of such a delay for the 800 ppm group (see figure 2).

Table 10 – Effects on male preputial separation.

Sex & parameter	Males / Preputial separation			
Dose [ppm]	**0**	**300/150**	**800/400**	**1600/800**
Pups examined [N]	38	40	39	39
Days to criterion	42.0	41.8	43.7**	47.9**
Historical control range [d]	39.7 - 42.5			
Body weight at criterion [g]	176.6	174.0	180.5	199.5**
Historical control range [g]	156.5 - 181.0			

* = P ≤ 0.05, ** = P ≤ 0.01 (Dunnett-test, two-sided)

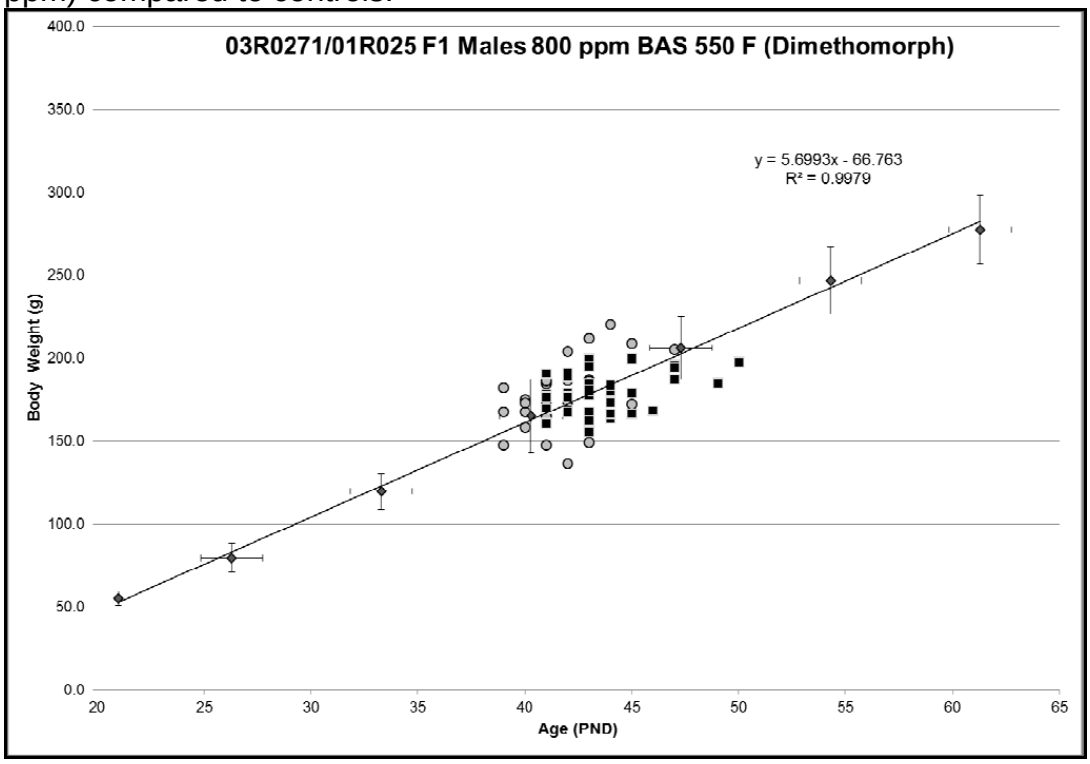
Figure 1 – Individual data on preputial separation for males of the high dose group (1600 ppm) compared to controls.



■ day of preputial separation – high dose group

○ day of preputial separation – control group

Figure 2 - Individual data on preputial separation for males of the mid dose group (800 ppm) compared to controls.



day of preputial separation – mid dose group

day of preputial separation – control group

This effect seen at the high dose is just a transient effect as all males reach sexual maturity and is not accompanied by other effects related to reproductive performances, such as changes is sperm parameters, changes on hormonal levels (testosterone and LH) and permanent changes in male sexual organs.

According to the results of the 2-generation study, **no impairment of mating behaviour, reproductive performance or physiology was observed.**

Concerning the gestation duration, a slight effect was reported in the 2-generation study (see table 11), this effect was statistically significant only with a significance of p<0.05, in the F1 and F2A generation but **not** in the F2B generation.

Table 11 – Summary of pregnancy duration in the 2-generation study

Parameter		**Dose level (ppm)**			
		0	**100**	**300**	**1000**
Gestation duration (d)	F1	22.0 +/- 0.3	22.1 +/- 0.3	21.9 +/- 0.4	**21.8 +/- 0.4***
ND (#)		-	1	3	1
21 days (#)		1	-	3	4
22 days (#)		21	23	20	18
23 days (#)		1	2	1	-
Gestation duration (d)	F2A	21.9 +/1- 0.4	21.9 +/- 0.3	22.1 +/- 0.3	**21.7 +/- 0.5***
ND (#)		-	-	-	-
21 days (#)		2	2	-	7
22 days (#)		16	19	18	17
23 days (#)		1	-	2	-
Gestation duration (d)	F2B	21.9 +/- 0.2	22.2 +/- 0.5	21.9 +/- 0.3	21.8 +/- 0.4
ND (#)		-	-	-	-
21 days (#)		1	1	1	3
22 days (#)		18	12	11	15
23 days (#)		-	4	-	-

*Statistically significant p<0.05 (ANOVA)
ND = sperm could not be detected, therefore day 0 not determined.

The ANOVA+Student's Newman Keuls test was used for statistical analysis in the study report. For 3 dose groups and one control group this test does not hold the family wise error rate of alpha=5%.
The Dunnett test, however, holds the family wise error rate of 5% and has been developed for simultaneous comparison against the control group. Therefore, the Dunnett test is considered the better choice for the analysis of this type of data and as a consequence is also used for the extended 1-generation study for multiple parameters including duration of gestation.

The results of the re-evaluation of the 2-generation study data using the Dunnett test (two sided) are given in the Table 12, showing **no statistical difference** between any treated groups compared to controls.

Table 12 - Re-evaluation of the duration of pregnancy (Dunnett test (two-sided))

Statistical analysis		**Dose level ppm**			
		0	**100**	**300**	**1000**
Mean	F1	22.00	22.08	21.92	21.82
SD		0.30	0.28	0.41	0.39
N		23	25	24	22
%DEV		0	0	-0	-1
SIG					
pvalue			0.7653	0.7489	0.1999
	F2A				
Mean		21.95	21.90	22.10	21.70
SD		0.40	0.30	0.31	0.47
N		19	21	20	23
%DEV		0	-0	1	-1
SIG					
pvalue			0.9696	0.4491	0.0900
	F2B				
Mean		21.95	22.18	21.92	21.83
SD		0.23	0.53	0.29	0.38
N		19	17	12	18
%DEV		0	1	-0	-1
SIG					
pvalue			0.1832	0.9930	0.6947

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.

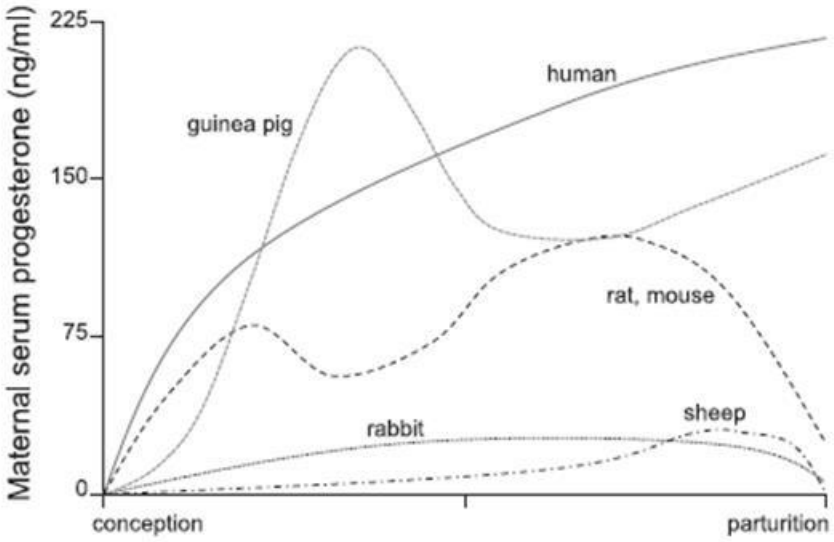
Reduced gestation length cannot be linked to the overall toxicological profile of dimethomorph. Considering a potential MoA, reduced gestation length **cannot be linked** to anti-androgenic properties: very potent anti-androgens (e.g. Vinclozoline and Flutamide) did not show any effects on pregnancy duration. In the absence of other potential MoA, it can be confirmed that this effect is not relevant.

Moreover, hormonal control of gestation duration in rats is completely different compared to humans. Differences between the species have been outlined by Mitchell & Taggert (2009). The main differences are shown in table 13 and figure 3:

Table 13: Hormonal control of parturition in rats versus humans

Rat	**Human**
Gestation duration 22±1 days	Gestation duration 226±14 days
Standard litter size 10±	Standard litter size 1
Pregnancy highly dependent on progesterone	Progesterone blood levels remain high at birth
Progesterone produced in Corpus luteum	Progesterone produced by placenta from week 6-8 (guinea pig week 4) of pregnancy
Increased levels of PGF_{2a} lead to luteolysis and rapid decrease of blood progesterone levels	Mechanisms of functional progesterone withdrawal discussed but not proven so far
Increase of blood estrogen levels at parturition	High placental aromatase activity
Estrogen synthesis in ovaries, no placental aromatase activity	Blood estrogen levels unchanged at parturition
	Fetal membranes can convert estrogen of low activity to more active forms and metabolise progesterone to less active forms

Figure 3 – Maternal serum concentrations of progesterone through gestation



from Mitchell&Taggert 2009 Am J Physiol Regul Integr Comp Physiol 297: 525-545

In rats gestation is ended by a sharp decline in progesterone serum levels accompanied by increasing levels of estrogen at parturition while in humans no such decline in progesterone and decrease in estrogen occurs.
The profound species differences between rats and humans regarding control of parturition has already been acknowledged during the evaluation of data on Fenarimol by the Scientific Committee on Plants in 1999. In the Opinion of the Scientific Committee on Plants regarding the possible inclusion of Fenarimol in Annex 1 of Directive 91/414/EEC concerning the placing of plant protection products on the market (SCP/FENARI/005-FINAL) it is stated: ”It also concluded that the effects of fenarimol on parturition in rats could be considered as not relevant for human risk assessment.”

Based on the considerations regarding onset of parturition and hormonal control of gestation, human relevance of a marginal change in gestation length in rat studies with Dimethomorph has to be doubted.

Thus, even in the unlikely case that this is a treatment related effect it would be of low relevance to humans and therefore not relevant for classification.

I.4 - Effects relevant for classification of Dimethomoph

- **For developmental toxicity:**

Developmental effects seen in the extended 1-generation are limited to the high dose tested males and consisted of **decreased pup weights secondary to maternal toxicity** and **slight decrease in AGD and AGI**.

No effects were observed on **pup survival or sex ratio** or on other very sensitive developmental landmarks as **nipple retention**. **No malformation**s were seen in **male reproductive organs.**

Moreover, **no malformation**s were observed in the **rat and rabbit developmental studies.**

Based only on a weak decrease of AGD/AGI in males, transient parameter based on that no different in the sex ratio occurred, on a weight of evidence basis as no other developmental effects were observed, a classification in Cat. 1B for development is not warranted.

- **For fertility:**

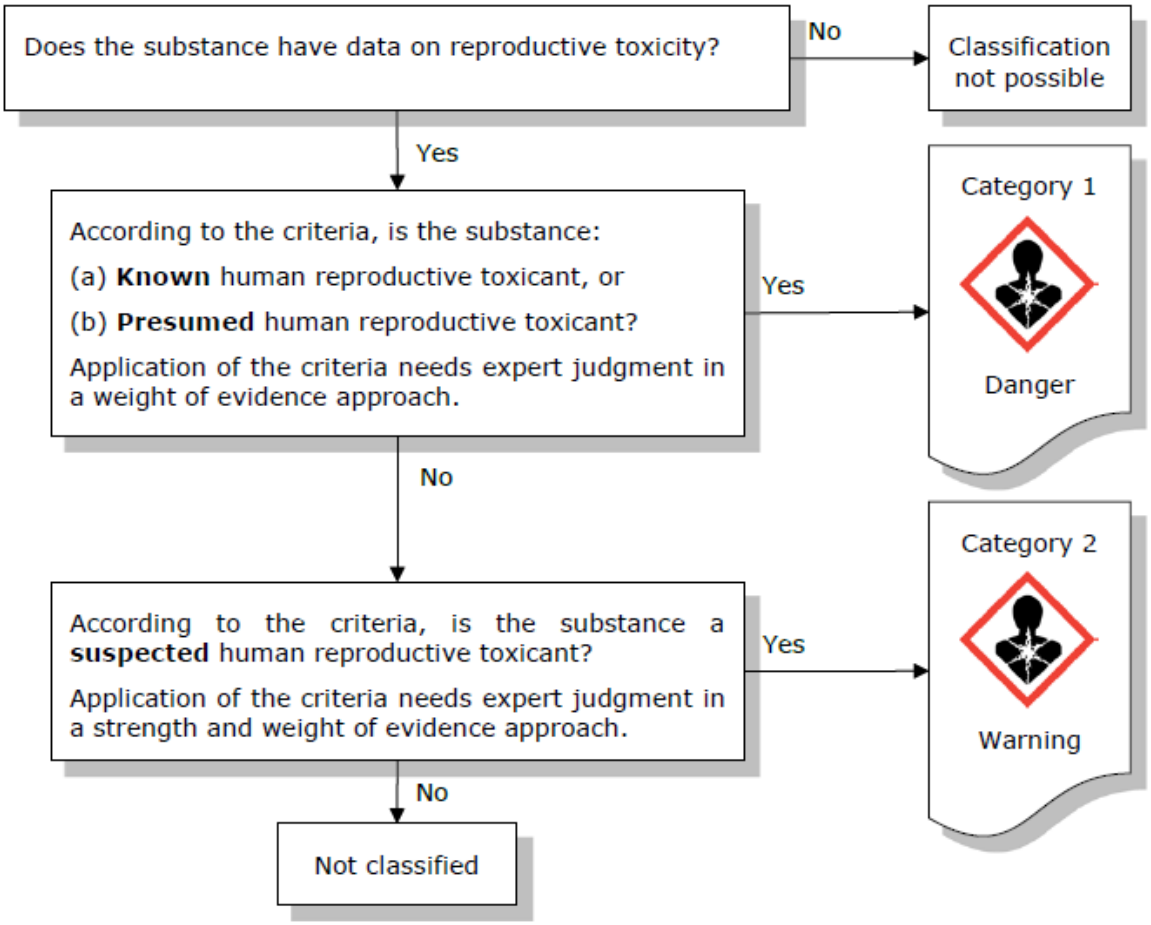
Fertility effects seen in the extended 1-generation study were limited to the high dose group in presence of maternal toxicity. The main effect is considered to be **a delay of preputial separation** in males at a high dose only and possibly confound by decreased body weights. This effect was not accompanied with changes in any other fertility parameters: **no changes in sperm parameters**, **no changes** in male pup **hormonal levels and no effects on reproductive performance** in the extended 1-generation and the 2-generation study.

A slight decrease was observed on prostate weight and seminal vesicle weight but **without histopathological correlate**.

Based only on delayed preputial separation accompanied by body weight effects and without any persistent effects on sperm, hormonal levels and reproduction performances, a classification in Cat. 1B for fertility is not warranted.

II - Comparison with recent ECHA classifications

In Regulation (EC) No 1272/2008, it is clearly stated that application of the classification criteria “needs expert judgement in a strength and weight of evidence approach”.



In order to identify if effects observed with dimethomorph (slight changes in AGD/AGI in males and delayed preputial separation in males at the top dose of the extended 1-generation study) would trigger a classification Cat. 1B for both development and fertility, a comparison with 226 substances for which a legal classification was proposed by ECHA (competent authority for classification) was performed.

In this evaluation, substances with effects on AGD/AGI and delayed sexual maturation (preputial separation and/or vaginal opening) were selected and analysed for the main effects triggering a possible classification as Repr. 1B or 2.

There were 5 examples found of substances classified as Repr. 1B where effects on ADG/AGI or sexual maturation supported the classification. Excerpts of these RAC opinions are included in the annex 1.

Table 14: Overview of RAC decisions for substances with classification as Repr. 1B and for which AGD/AGI and sexual maturation effects were mentioned in the RAC opinions

Compounds	**Effects triggering the classification**	**Conclusions**
Dicyclohexylphthalate *RAC opinion Dec 4th, 2014:* H360D	Proposal to classify in Cat 1B for development was mainly based on: - Reduced AGD and **increase incidence of areola mammae** in male pups - Prolonged preputial separation and **hypospadias** associated with small testis - **Testicular tubular atrophy** and **atrophic tubules in prostate**	No classification for fertility Effects on AGD and preputial separation in males were observed concurrently with severe malformations of the male reproductive tract and were not the main driver for classification
Perfluorononan (PFNA) *RAC opinion Sept 12th, 2014:* H360fD and Ammonium pentadecafluorooctanoate (APFO) (read across) *RAC opinion Dec 2nd, 2011:* H360D, H362	Proposal to classify in Cat 1B for development was mainly based on: - **Reduced pup viability** - **Full litter resorption** Proposal to classify in Cat 2 for fertility was mainly based on: - **Changes in sperm parameters** - **Changes in hormonal levels** - **Histopathological changes** in the male reproductive organs.	Delayed onset of puberty was observed together with effects on pup viability and was not the main driver for classification. Classification in Repr. 2 for fertility was mainly based on severe changes of the male reproductive organs associated with changes in hormonal levels and sperm parameters
Phenol, dodecyl-, branched [1], Phenol, 2-dodecyl-, branched, Phenol, 3-dodecyl-, branched, Phenol, 4-dodecyl-, branched; Phenol, (tetrapropenyl) derivatives [2] (accelerated vaginal patency supporting classification) *RAC opinion Dec 5th, 2013*: H360F	Proposal to classify in Cat 1B for fertility was mainly based on: - Effects on male and female **reproductive parameters** - **Reduced fertility**	Delayed onset of puberty was observed concurrently with severe effects on reproduction and was not the main driver for classification.
Di-n-hexyl phthalate (DnHP) *RAC opinion Sept 13th, 2011*:	Proposal to classify in Cat 1B for development was mainly based on: - **Teratogenicity**	Delayed onset of puberty and reduced ADG were observed together with teratogenicity and embryo-

Compounds	Effects triggering the classification	Conclusions
H360FD	- **Embryo-mortality** Proposal to classify in Cat 1B for fertility was mainly based on: - Severe adverse effects on **male sexual function** and **male sexual organs** - **Altered fertility**	mortality and were not the main drivers for classification in Cat 1B for development. Classification in Cat 1B for fertility was mainly based on severe changes of the male reproductive organs associated with altered fertility.

In conclusion, when compared to the 226 substances with ECHA classification according to Regulation (EC) No 1272/2008, RAC has never assigned a classification to Cat. 1B based only on AGD/AGI and/or delay in sexual maturation.

For classification in Cat 1B for fertility, the main drivers for classification were:
- Decreased fertility
- Decreased sperm count
- Decreased litter size
- Decrease in fertility index
- Decrease in mating index
- Severe effects on sexual organs including histopathological changes

For classification in Cat. 1B for development, the main drivers for classification were:
- Stillbirth
- Post-natal mortality
- Malformations
- Effects on sexual maturation associated with malformations of the male reproductive tract.

Statistics of proposed classifications in relation to AGD/AGI and sexual maturation is given in table 15.

Table 15 – Summary of classifications for which AGD/AGI and sexual maturation effects were mentioned in the RAC opinions

226 Substances with RAC opinion published by ECHA	**No. of substances with legal classification**		
	Repr. 1B	**Repr. 2**	**not classified for reprotox**
Substances classified	44	36	151
AGD and/or sexual maturation only effect triggering classification	0	0	-
AGD and/or sexual maturation mentioned in context of classification (classification in Repr. 1B driven by reduced fertility, pup mortality or malformations)	5	1	0
AGD and/or sexual maturation not considered for classification	2	3	5

None of the severe effects that were so far triggering a classification as Repr. 1B for fertility or development occurs for dimethomorph. Therefore, compared to available harmonized classifications, a strong weight of evidence demonstrates that classification as Repr. 1B is clearly disproportionate based on the slight effects seen with dimethomorph.

III - Comparison with recent EFSA conclusions

A comparison with a recent EFSA classification proposal was made based on the example of another active substance notified by BASF. A full evaluation of EFSA proposals has not been performed like what has been done for ECHA conclusions, however, the recent evaluation of the active substance iprodione is considered very helpful for the comparison with dimethomorph.

Like dimethomorph, iprodione is currently evaluated under AIR3. The classification proposal for iprodione according to the EFSA journal published November 28th 2016 is **Cat. 2 for fertility and development (H361fd)**. Details of the results with iprodione can be found in Annex II.

In table 16 effects observed with iprodione are compared to those seen with dimethomorph, marked in **bold** are effects which triggered the classification for reproductive toxicity of iprodione.

Table 16: Comparison between effects induced by Iprodione and Dimethomorph

	Iprodione	**Dimethomorph**
Maternal toxicity	Decreased food consumption and body weight gain at the high dose	Decreased food consumption, body weight and body weight gain at the high dose. Hepatotoxicity.
Effects on development	Effect on AGD **Nipple retention** at the high dose **Malformation** (umbilical hernia in rabbit)	Effect on AGD/AGI at the high dose. Reduced pup body weight at the high dose related to maternal toxicity.
Effects on fertility	Effect on preputial separation at the two top doses. Effects on sexual organs in rat and mouse with histopathological finding: - **Leydig cell hyperplasia** - **Atrophy of the seminiferous tubules** - **Atrophy of seminal vesicles and prostate** - **Decreased ovary weight** - **Decreased number of corpora lutea** - **Atrophy of the uterus** Effects on **sperm parameters** and decrease in **testosterone level** in the 2-generation study.	Effect on preputial separation at the top dose. Decrease of prostate weight and seminal vesicles at the high dose without histopathological correlate.

While effects on maternal toxicity are comparable or slightly less than what was seen with Dimethomorph effects on AGD/AGI combined with nipple retention and preputial

separation are more pronounced for Iprodione. In addition, Iprodione shows histopathological changes in reproductive organs and effects on sperm parameters.

In conclusion, the effects for iprodione are more pronounced than for dimethomorph and additional effects of higher severity are seen. The classification proposal for iprodione as Repr. Cat. 2 H361 fd issued only a few months ago underlines the disproportionality of the classification proposal for dimethomorph as Repr.1B H360 FD.

Conclusion on the classification proposal for Dimethomorph

In the extended 1-generation with dimethomorph, slight effects are observed in F1 male rats at the highest tested dose, in presence of moderate maternal toxicity (decreased body weight, body weight gain and hepatotoxicity).

These effects on F1 offspring were limited to:

- Decreased body weight of the pups
- Slight decrease in AGD/AGI
- Delay in preputial separation

These observations may be in line with the anti-androgenic properties seen *in vitro,* however, as no other fertility and developmental parameters were affected, the effects are not severe enough to warrant classification Cat. 1B for both fertility and development.

Moreover, a comparison with recent ECHA decisions and EFSA classification proposals clearly demonstrated that this kind of effects only seen in isolation do not trigger classification in Cat. 1B.

Therefore, the notifier considers that the RMS' proposal to classify dimethomorph in Cat. 1B for both fertility and development is clearly disproportionate compared to other substances.

Annex 1:
Excerpts from RAC opinions on 5 substances with classification as Repr. 1B and effects on ADG/AGI and sexual maturation

- **Dicyclohexylphthalate RAC opinion Dec 4th, 2014: H360D**

"The experimental animal data for DCHP effects on development indicated a reduced AGD and an increased incidence of areola mammae in male pups. These effects were reported in three independent studies in the absence of marked maternal toxicity. In addition, prolonged preputial separation and hypospadias associated with small testis was described in one of the studies. The adverse effects observed …in male reproductive organs, including testicular tubular atrophy and atrophic tubules in the prostate, occurred after *in utero* exposure and were considered as supportive evidence for developmental effects. Taken together, all these effects, which were observed following parental exposure in the absence of marked maternal toxicity, provide clear evidence of an adverse effect on development in the absence of other toxic effects. These effects have also been shown to occur following exposure to various transitional phthalates and are consistent with an anti-androgenic action of DCHP, which is considered relevant to humans. Classification as Repr. 1B is therefore warranted.
The experimental animal data available did not show a clear adverse effect of DCHP on sexual function and fertility. No effects on fertility parameters were reported in a 2-generation study performed according to OECD TG and GLP. Effects on the male reproductive organs such as testicular atrophy, Sertoli cell vacuolisation, epididymis without sperm and/or abnormal sperm in the tubules and a decreased weight of the prostate as well as atrophic prostate tubules, were observed following in utero exposure to DCHP."

Proposal to classify in Cat 1B for development was mainly based on:

- Reduced AGD and **increase incidence of areola mammae** in male pups
- Prolonged preputial separation and **hypospadias** associated with small testis
- **Testicular tubular atrophy** and **atrophic tubules in prostate**

No proposal for fertility classification

Overall, effects on AGD and preputial separation in males were observed concurrently with severe malformations of the male reproductive tract and were not the main driver for classification

- **Perfluorononan (PFNA) RAC opinion Sept 12th, 2014: H360fD and**
  Ammonium pentadecafluorooctanoate (APFO) read across RAC opinion Dec 2nd, 2011: H360D, H362

"Taking into account
- minor effects (small reductions in sperm motility and sperm count in epididymis of F0 males, but not in F1 males) …;
- increase in serum testosterone levels, decrease in serum estradiol levels and increased frequency of spermatogenic cells with apoptotic features in rats exposed by gavage to 5 mg PFNA/kg/d (Feng *et al.*, 2009);
- reduced plasma testosterone concentrations, increased frequency of abnormalities in sperm morphology and vacuolated cells in the seminiferous tubules …
- the supporting preliminary human data,

RAC is of the opinion that classification of PFNA, PFN-S and PFN-A as Repr. 2, H361f (Suspected of damaging fertility) is warranted."

"Taking into account that exposure to PFNA in mice during gestation reduces pup viability, pup body weight gain, delays puberty as well as onset of eye opening, increases both dam and pup absolute and relative liver weight, and induces full litter resorptions/loss at high doses as well as that the developmental toxicity of PFNA in mice are qualitatively and quantitatively similar to developmental toxicity of PFOA (reduced pup viability, full litter resorption and delay in the onset of eye opening)
RAC is of the opinion that PFNA and its ammonium and sodium salts should be classified as Repr. 1B, H360D."

Proposal to classify in Cat 1B for development was mainly based on:
- **Reduced pup viability**
- **Full litter resorption**

Proposal to classify in Cat 2 for fertility was mainly based on:
- **Changes in sperm parameters**
- **Changes in hormonal levels**
- **Histopathological changes** in the male reproductive organs.

Overall, delayed onset of puberty was observed together with effects on pup viability and was not the main driver for classification. Moreover, classification in Repr. 2 for fertility was mainly based on severe changes of the male reproductive organs associated with changes in hormonal levels and sperm parameters.

- **Phenol, dodecyl-, branched [1], Phenol, 2-dodecyl-, branched, Phenol, 3-dodecyl-, branched, Phenol, 4-dodecyl-, branched; Phenol, (tetrapropenyl) derivatives [2] (accelerated vaginal patency supporting classification) RAC opinion Dec 5th, 2013: H360F**

" - Reduced epididymal sperm count and prolongation of oestrous cycle at a dose of 75 mg/kg in the two-generation reproductive study in rats…

- Reduced number of pups born in the F2a generation exposed to a dose of 75 mg/kg…
- Reduced proportion of animals copulating when cohabited, reduced litter size, alterations in number of corpora lutea, prolongation of oestrous cycle and reduced epididymal sperm count in animals exposed at 125 mg/kg in the one-generation study in rats … "
- Altered onset of "sexual maturation in female animals that is reported in the two-generation study and in the female pubertal assays.

The mechanistic information further suggests that TPP has weak estrogenic and androgenic activity.
Reduction of fertility index: 13% at 125 mg/kg bw/d"

Proposal to classify in Cat 1B for fertility was mainly based on:

- Effects on male and female **reproductive parameters**
- **Reduced fertility**

Overall, effects delayed onset of puberty was observed concurrently with severe effects on reproductive effects and were not the main driver for classification.

- **Di-n-hexyl phthalate (DnHP) RAC opinion Sept 13th, 2011 H360FD**

"DnHP induced a significant and dose-related decrease in the anogenital distance and increased incidence of thoracic aerolas and/or nipples of male rat foetuses at all doses"
…..
"Overall, based on animal studies:
- DnHP dose-dependently reduces the fertility of adult mice in a continuous breeding study;
- Teratogenicity has been described in rats with numerous malformations at higher doses. Specifically, DnHP impacts the male reproductive system with permanent alteration of testis, epididymis and seminal vesicle weight and histopathology in rats exposed *in utero*, likely leading to impairment of fertility after repeated exposure;
- DnHP induces embryo-mortality (decrease in litter and pups production) in absence of maternal body weight modification in mice and rats by oral route;
- Embryo-toxicity has been described in rats with reduced pup body weight, delayed ossification and increased incidence of skeletal variants.
It is concluded that the data provided in the report provide clear evidence of teratogenic and foeto-toxic effects of DnHP. They also provide clear evidence of an adverse effect on male sexual function and fertility. Moreover, there is no mechanistic evidence that could lead to think that these effects are not relevant for human."

Proposal to classify in Cat 1B for development was mainly based on:
- **Teratogenicity**
- **Embryo-mortality**

Proposal to classify in Cat 1B for fertility was mainly based on:
- Severe adverse effects on **male sexual function** and **male sexual organs**
- **Altered fertility**

Overall, effects on delayed onset of puberty and reduced ADG were observed together with teratogenicity and embryo-mortality and were not the main driver for classification in Cat 1B for development.
Classification in Cat 1B for fertility was mainly based on severe changes of the male reproductive organs associated with altered fertility.

Annex 2:
Results relevant for classification of Iprodione

Iprodione is evaluated under AIR3 (notifier BASF). The classification proposal according to EFSA journal published November 28th 2016 is Cat. 2 for fertility and development (H361fd).

"In reproductive toxicity studies, fertility and overall reproductive performance was not impaired; however, increased abnormal sperm was observed in F1. Iprodione has been shown to induce developmental toxicity, i.e. delayed onset of male puberty and persistence of areolas in the two-generation study and umbilical hernia in the rabbit developmental toxicity study. …..
On the basis of the reassessment of reproductive toxicity studies, new mechanistic data and taking into account guidance on the application of the CLP Criteria (ECHA, 2015) the adverse effects observed in the reproductive toxicity studies and adverse effects in reproductive organs in other toxicity studies, as well as the results of the mechanistic studies, suggest that classification regarding reproductive toxicity would be required for iprodione as 'toxic for reproduction category 2 (H361df)"

Annex 2.1 - Effects relevant for developmental classification of Iprodione

For maternal toxicity, a slight effect was seen on maternal food consumption and body weight gain at the top dose of the 2-generation study.

No statistically significant effects were seen on pup body weight.

A slight decrease in AGD was observed in the high dose, **nipple retention** was also seen at this dose level and was considered as a **significant effect for classification (see table 1).**

Table Annex 2.1 – Effects on AGD/AGI and nipple retention with Iprodione

Dose [ppm]	**0**	**300**	**750**	**1500**
- Pup Observation				
AG-Distance males (mm)	3.09 (100%)	3.15 (102%)	3.04 (98%)	2.95 (95%)*
AG-Index males	0.47 (100%)	0.47 (100%)	0.48 (101%)	0.45 (96%)
Areolas/Nipples (day 12 PND)	67,0%	78,3%	57,4%	77,5%
Areolas/Nipples (day 20 PND)	0	0	1	4 (1 litter)

Moreover**,** malformation (umbilical hernia) were observed in the developmental study in rabbit, triggering also a classification for developmental toxicity.

Annex 2.2 – Effects relevant for fertility classification of Iprodione

In the 2-generation study performed with Iprodione, effects on preputial separation were also observed (see table 2).

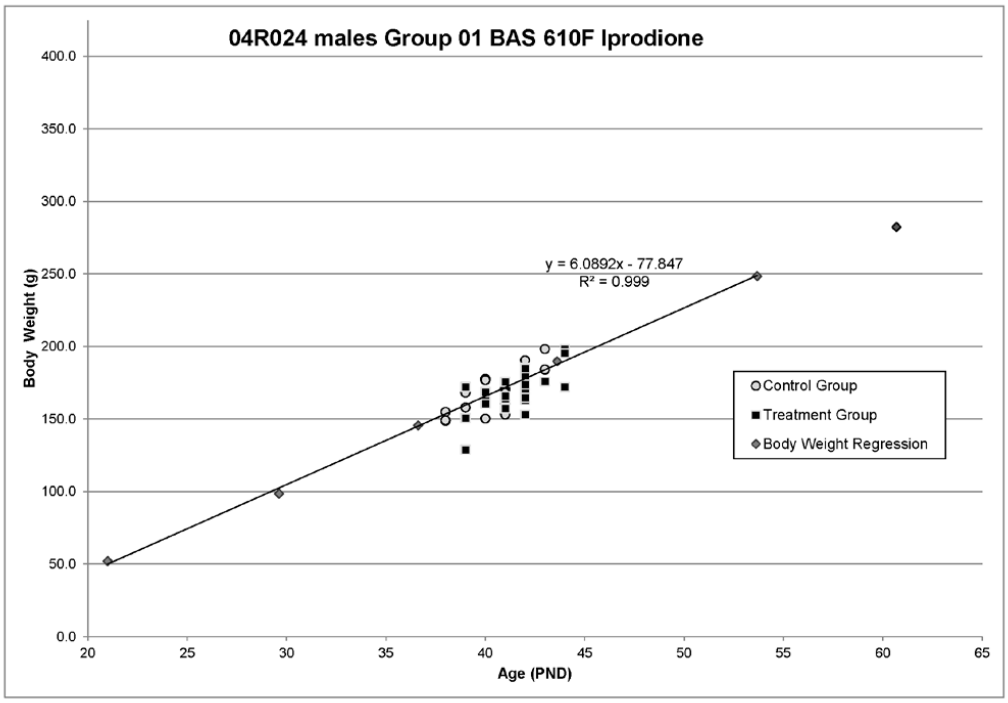
Table Annex 2.2 – Effects on preputial separation observed with Iprodione

	Males F1				**Males F2**			
Dose [ppm]	**0**	**300**	**750**	**1500**	**0**	**300**	**750**	**1500**
	Preputial separation							
Animals per dose	25	25	25	24	25	25	25	25
- Days to criterion	40.6	41.4	44.8**	48.2**	40.1	41.8**	45.1**	47.9**
- Body weight at criterion [g]	169.5	170.2	185.7**	198.4**	168.4	168.7	191.1**	196.8**

* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnett-test, two-sided)

Effects were not only seen at the highest dose but were also significant in the mid dose. When comparing individual to the control animals, there is a clear shift for a longer preputial separation for the 750 ppm group (figure 1) and also for the higher dose (figure 2).

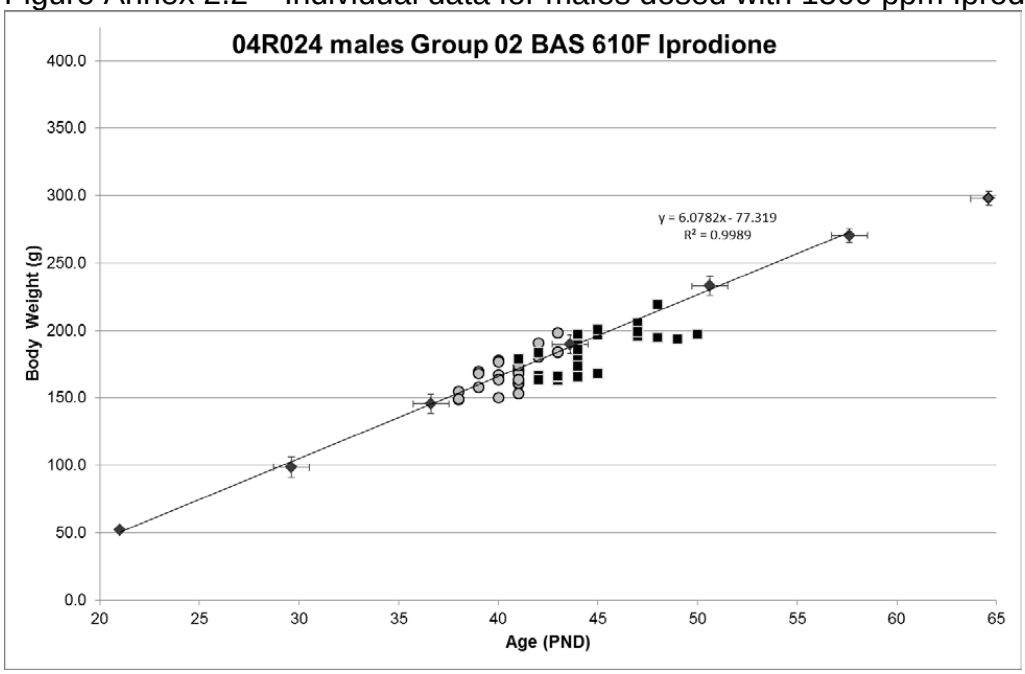
Figure Annex 2.1 – Individual data for males dosed with 750 ppm Iprodione



■ day of preputial separation – 750 ppm group

○ day of preputial separation – control group

Figure Annex 2.2 – Individual data for males dosed with 1500 ppm Iprodione



■ day of preputial separation – 1500 ppm group

○ day of preputial separation – control group

Moreover, other effects related to fertility were observed in repeated dose toxicity studies and also in the 2-generation study with iprodione:

- 90-day rat study:
  Leydig cell hyperplasia, atrophy of the seminiferous tubules, seminal vesicles and prostate atrophy, decrease ovary weight, decrease number of corpora lutea, uterus atrophy
- 28-day mouse study:
  increase in Leydig cell hyperplasia
- 90-day mouse study:
  decrease ovary weight, decrease number of corpora lutea, uterus atrophy
- 2-generation study in rats:
  weak effects on sperm parameters and decrease in testosterone levels.

Overall, the proposed classification for iprodione was based on severe effects on reproductive organs accompanied by effects on AGD, nipple retention and delay in preputial separation for fertility.

The main driver for developmental classification was malformation seen in the rabbit developmental study. **Repr. Cat. 2 H361 fd** is proposed by EFSA.