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Comments on the CLH Report Proposal on propyl 4-hydroxybenzoate [Cas-No. 94-13-3]

Clariant Produkte (Deutschland) GmbH – Comments on the CLH Report Proposal for Harmonised Classification and Labelling – Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2, Substance Name: propyl 4-hydroxybenzoate; EC number: 202-307-7 CAS number: 94-13-3; Dossier Submitter – Belgium (FPS Public Health, Food Chain Safety and Environment DGEM/ Department of Product Policy and chemical Substances / Management of Chemical Substances)

Clariant Produkte (Deutschland) GmbH is the lead registrant company of propyl 4-hydroxybenzoate. The opinion of Clariant Produkte (Deutschland) GmbH on the classification proposal is as follows:

- Based on the scientific evidence, Clariant Produkte (Deutschland) GmbH does not agree with the proposal for classification of propyl 4-hydroxybenzoate as a Reproductive toxicant Category 2 (Fertility- H361F) based on supposed effects on sperm motility and morphology, as all the values from the most conclusive Extended One-Generation Reproductive Toxicity Study (EOGRTS) are within the range of historical control data, are not statistically significant and are not dose dependent. More importantly, no effects were observed in any functional parameters in male reproduction and fertility up to 1000 mg/kg bw per day.
- Based on the scientific evidence, Clariant Produkte (Deutschland) GmbH does not agree with the proposal for classification of propyl 4-hydroxybenzoate as a Reproductive toxicant Category 2 (Development- H361D) based on supposed anogenital distance (AGD) changes in male rats (only F2 generation), as all values from the most conclusive EOGRTS are within the range of historical control data and no dose dependency was observed in these effects. More importantly, this effect was especially not confirmed in F1 Cohort 1B pups revealing that these effects are not test item related but due to biological variation. Gross pathological and histopathological examination did not show any effects on male reproductive organs up to 1000 mg/kg bw per day. In the absence of such structural or functional reproductive effects, an isolated consideration of AGD data is scientifically not justified as such findings should be present due to the strong association of short male AGD with genital malformations at birth and reproductive disorders in adulthood. Therefore, the effect on AGD is not considered test item related.
- Based on the scientific evidence, Clariant Produkte (Deutschland) GmbH does not agree with the proposal for classification of propyl 4-hydroxybenzoate as a Reproductive toxicant Category 2 (Development- H361D) based on marginally post implantation loss. All the values are within the range of historical control data, are not statistically significant and are not dose dependent. More importantly, this effect was especially not confirmed in the cohort 1B revealing that these effects are not test item related and due to biological variation at the values are within historical control data.

- The opinion of Clariant Produkte (Deutschland) GmbH is supported by a recently finalized SCCS reevaluation (SCCS/1623/20) on propyl 4-hydroxybenzoate conducted following the European Commissions ‘Call for data on ingredients with potential endocrine-disrupting properties used in cosmetic products’. For this reevaluation the original study report of the EOGRTS was provided by industry and considered by the SCCS. In its opinion the SCCS concludes that the NOAEL for reproductive and developmental toxicity can be set at 1000 mg/kg bw per day.
- Clariant Produkte (Deutschland) GmbH notes that the key studies central to the CLH dossier are the Extended One-Generation Reproductive Toxicity Study (EOGRTS) (Study No 176898, 2021, Study owner Clariant Produkte (Deutschland) and a historical non-OECD/non-GLP study published by Oishi et al 2002. The EOGRTS as discussed above is an OECD TG and GLP conform study and can therefore be considered as the most valuable and valid information source for the evaluation on reproductive toxicity of propyl 4-hydroxybenzoate. Regarding the study of Oishi et al. (2002) however, identified limitations do not allow its unrestricted use for a scientific and robust evaluation. For more details, please refer to chapter 2.2.3.
- Clariant Produkte (Deutschland) GmbH notes that a re-evaluation of the concerns raised by the MS Belgium in the CLH dossier, done by the CRO BSL BIOSERVICE who performed the study (Annex II), is also confirming the absence of any test item related reproductive toxic effects and confirmed that no adverse effect on parameters regarding sexual function, fertility and/or development can be derived based on the findings in the EOGRTS.
- Clariant Produkte (Deutschland) GmbH notes that the CLP criteria as Repro. Cat 2 for propyl 4-hydroxybenzoate are also not met as no adversity of the postulated effects on sexual function, fertility and/or development in parental animals and their offspring could be demonstrated based on the available experimental data. In this respect it must be mentioned that an isolated consideration of statistically non-significant effects on single endpoints, which are moreover within the range of historical control data, is not justified for classification as developmental and reproductive toxicity. This view is supported by the CLP regulation itself which states that “*if in some reproductive toxicity studies in experimental animal the only effects recorded are considered to be of low or minimal toxicological significance classification may not necessarily be the outcome*” (CLP 3.7.2.3.3).

The above opinion is supported by detailed comments which are provided hereafter in five parts:

Part 1- Short Summary of CLH proposal on propyl 4-hydroxybenzoate

Part 2- Scientific evaluation of potential effects of propyl 4-hydroxybenzoate

Part 3- Overall conclusion on developmental and reproductive toxicity

Part 4- CLP and Scientific Justification for No Classification

Annex I- Summary of EOGRTS on propyl 4-hydroxybenzoate

Annex II- Re-evaluation of the EOGRTS by CRO BSL BIOSERVICE

1. Short Summary of CLH proposal on propyl 4-hydroxybenzoate

In the CLH-dossier provided by Belgium as the evaluating MS a classification as Repr. 2, H361d,f is viewed as warranted for propyl 4-hydroxybenzoate based on following effects:

- lower anogenital distance (AGD) in pups and increased post-implantation loss as postulated **adverse effect on development**

- effects in sperm motility and morphology in the absence of clear general toxicity as postulated **adverse effect on fertility**

The registrant does not agree with the above position provided by the Belgium MS. Instead, assessing the total weight of evidence from all available studies on propyl 4-hydroxybenzoate reveals that all findings are well within the range of historical control data and thus reflect biological variation rather than a test item related effect. Importantly, neither adverse effects on sexual function and fertility in adult males and females nor developmental toxicity in the offspring could be evidenced and thus the toxicological information do not support a classification of propyl 4-hydroxybenzoate as Repr. 2 H361d,f.

In the following a scientific evaluation of the concerns raised by the CLH-dossier submitter Belgium regarding adverse effects on development and fertility is given.

2. Scientific evaluation of potential effects of propyl 4-hydroxybenzoate

2.2. Adverse effects on development

The CLH-proposal raised a concern regarding developmental toxicity based on slightly lower anogenital distance (AGD) in pups and slightly increased post-implantation loss in an EOGRTS. However, Clariant Produkte (Deutschland) GmbH does not agree with the above position provided by the Belgium MS as all the evaluated values were in the range of historical control data and no dose dependency could be observed. Furthermore, the changes are not statistically significant and could not be revealed in both generations of the EOGRTS indicating that these effects are due to biological variability rather than test item related. In the following a scientific evaluation of these parameters is given.

The aim of EOGRTS was to assess possible adverse effects of the test item propyl 4-hydroxybenzoate via oral administration (gavage) after repeated exposure during all phases of the reproductive cycle including pre and postnatal development with sexual maturation, the integrity of the male and female reproductive systems and systemic toxicity in males, pregnant and lactating females and young/adult offspring (development, growth, survival, and functional endpoints). In a single study, it tests parental (P) fertility and reproductive function, and offspring (F1) development through sexual maturity, including assessment of sexual landmarks (Cohort 1), nervous system - neuropathological and behavioural endpoints (Cohort 2), immune function (Cohort 3), and effect on F2 generation by mating of F1 offspring if there are indications of potential adverse effects on

F1 offspring. The rats were treated with 0, 100, 300 and 1000 mg/kg bw per day. A detailed description of the results is given in Annex I.

Based on the Extended One-Generation Reproductive Toxicity Study after oral administration of propyl 4-hydroxybenzoate to male and female Wistar Rats the following conclusions can be made: General toxicity: Few mortalities/morbidities were randomly distributed throughout the majority of groups of parental animals and various cohorts. Based on histopathology the cause of death was not evident in most animals whereas in few, the cause of death could be related to the technical gavaging error and not due to systemic toxicity caused by the test item. There were no clinical signs of toxicological relevance observed in the treatment groups. Regarding reproductive and developmental toxicity there were no considerable differences in the length or sequence of oestrous cycle stages, duration of precoital interval and the duration of gestation of the parental generation and cohort 1A between the treatment groups and the control group. There were no signs of abortion or premature delivery, in litter parameters, i.e. number of still births, runts, total number of pups, sex ratio, number of live pups, weight of pups, survival index. Corpora lutea, implantation sites, percent preimplantation loss and post implantation loss in parental generation and cohort 1B were unaffected by propyl 4-hydroxybenzoate. There was no toxicological effect of the test item on reproductive indices (male mating index, female mating index, male fertility index, female fertility index, gestation index and live birth index) in parental generation and in cohort 1B. No test item related external findings were observed in the pups of this study. There was no toxicological effect on anogenital distance and nipple retention and sexual maturity parameters (i.e. vaginal opening and balano-preputial separation). There were no effects on sperm motility and morphology as well as for sperm head count of parental generation and cohort 1A males. Histopathologic ally, no effects on reproductive organs were detected. There was no indication of endocrine disruptive properties of the test item in this study. In the absence of indication of toxicity, the NOAEL for developmental and reproductive toxicity, neurotoxicity and immunotoxicity is determined as 1000 mg/kg body weight/day.

In a Prenatal Developmental Toxicity study according to OECD 414 and GLP, propyl 4-hydroxybenzoate was administrated by oral gavage to 52 males and 104 females (ECHA REACH dossier 2018). The doses evaluated were 0, 100, 300 and 1000 mg/kg body weight/day. No mortality was observed in the study and there were no clinical signs of toxicological relevance observed in the treatment groups.

The body weight, food consumption, prenatal, litter data and gross pathology of terminally sacrificed females remained unaffected in the treatment groups when compared to the controls. Furthermore, no treatment-related and toxicologically relevant external, visceral or craniofacial findings were observed in the high dose group and other treated groups. Findings of reduced ossification of some bones and few other skeletal findings were well within the historical control data range for this strain of rats and not considered to be a substance related effect. Generally delayed ossification is not regarded to persist postnatally and not associated with long-term consequences on survival, general growth and development and therefore is not considered to be adverse. No effects of propyl 4-hydroxybenzoate on females and foetuses were found at dose levels up to 1000 mg/kg body weight/day. The NOAEL for both maternal toxicity and foetal toxicity is considered to be 1000 mg/kg body weight/day in this study.

Despite the data generated from the EOGRTS the CLH-dossier submitter raised a concern regarding developmental toxicity based on some minor effects on AGD and post implantation losses. In the following a scientific evaluation of these two endpoints is provided.

2.2.1. Potential Effects on AGD

Male AGD, the distance between the anus and the external genitalia, is an androgen-sensitive endpoint of the masculinization. A short male AGD is strongly associated with genital malformations at birth and reproductive disorders in adulthood. A concern regarding the reduction of AGD in male and female pups was raised by the CLH-dossier submitter based on the data provided in the OECD 443. Based on the data from the EOGRTS in male pups from the parental generation, on PND 0 marginal shorter absolute but not relative AGD was observed only in the HD group (1000 mg/kg bw per day) when compared to the concurrent controls. It is important to note that AGD is influenced by the body weight of the animal and therefore, this needs to be taken into account when evaluating the data (OECD Guidance document No. 151) and a normalization using the cube root of body weight is recommended in TG 443. In case of propyl 4-hydroxybenzoate (parental generation) no statistically significant effect could be observed after normalisation of AGD to cube root of body weight. More importantly, no dose dependency was observed in these effects and all the values are well within the range of historical control data (Appendix I- Table 1) revealing that this effect is not considered to be test item related but due to biological variation.

In male pups from cohort 1B on PND 0, marginal statistically significant shorter absolute and relative AGD were observed in the HD group when compared to the concurrent controls. However, as all values were well within the normal range of historical control data, this observation is not considered toxicologically relevant. More importantly, these effects are not dose dependent which supports that the effects are not test item related but due to biological variation.

As already mentioned, a shorter AGD in male offspring after fetal exposure to anti-androgenic compounds can be considered as a sensitive biomarker for malformations in sexually mature animals such as hypospadias, ectopic testes, absent bulbourethral glands, or absent prostates (Bowman et al. 2013; Christiansen et al. 2008; Welsh et al. 2008, 2010). Due to the strong correlation between AGD and various reproductive disorders and malformations, an isolated consideration of AGD is therefore not appropriate. Especially in the case of propyl 4-hydroxybenzoate, where changes in male AGD were only minimal, well within the range of historical control data and not dose dependent, the concurrent lack of any functional impairment contradicts the assumption of an adverse effect. The results from the EOGRTS clearly demonstrate that *in utero* exposure to propyl 4-hydroxybenzoate up to the limit dose of 1000 mg/kg bw per day did not induce any morphological or histopathological abnormalities in male reproductive organs. Importantly, functional parameters such as fertility and mating index were also not affected after treatment with propyl 4-hydroxybenzoate up to 1000 mg/kg bw per day neither in parental, nor in F1 and/or F2 animals. Also worth noting is the fact that an anti-androgenic mode of action causes beside a reduction of AGD also an increase in nipple retention as well as malformations

in reproductive organs. As already pointed out, the effects on nipple retention did not confirm that the findings on AGD are due to an anti-androgenic mode of action as the nipple retention is upregulated in the parental but downregulated in the F1 generation. These deviating and, in particular, contradictory effects, scientifically support the view that an anti-androgenic mode of action can be excluded and that the minor changes on AGD (and nipple retention (NR)) can plausibly be based on biological variability and therefore is not considered to be an adverse or toxicologically relevant effect.

No effect of toxicological relevance was observed on nipple retention in the pups of any of the groups from parental and cohort 1B when compared with the respective controls. Group mean number of nipple retention in HD males from parental animals (F1) was statistically significantly lower than in controls whereas in males from cohort 1B (F2), nipple retention was statistically significantly higher when compared with the controls. Additionally, it has to be mentioned that the apparent higher incidence of nipple retention in the F1 generation is due to pups from just one single dam which supports the conclusion that this finding is incidental and not related. Due to the inconsistent nature of the responses no toxicological relevance is attributable to this observation. Moreover, these findings were not associated with any developmental or reproductive toxic effects in cohort 1B animals.

In the female pups from the parental generation, a minimal lower absolute and relative anogenital distance in LD, MD and HD groups was observed on PND 0 when compared with the controls. As all these differences were only very minor and all values were still within the range of historical control data, this is not considered to be toxicologically relevant. More importantly, since a reduction of AGD in females is without any toxicological relevance the dossier evaluator should have explained why the effects on female AGD were considered as adverse.

In the OECD Guidance Document No. 151 vinclozolin is described as a well-known reproductive/developmental toxicant with an androgen receptor antagonistic mode of action. In the F1 generation endpoints associated with the development of the male reproductive system such as genital abnormalities, delayed preputial separation, reduced reproductive organ weight were all altered in line with a reduced AGD. Additionally, some effects on the female reproductive system in the F1 generation were also observed such as reduced age at vaginal opening and oestrus cycle disturbance. This example shows that the effects on AGD are not isolated in nature but are strongly correlated with other effects on male or female reproductive organs. In case of an anti-androgenic mode of action, a consistent reduction in AGD and a consistent increased NR together with other malformations on male reproductive organ should be therefore observed. In case of propyl 4-hydroxybenzoate neither consistent effects on AGD and/or nipple retention, nor other changes in male or female reproductive organs or performance were detected.

Appendix I – Data and discussion regarding AGD based on EOGRTS

In the following a summary of male and female AGD and male NR of F1 and F2 pups are presented:

Summary Anogenital Distance and Nipple Retention – F1 Cohort 1B pups

Table 1 Summary AGD – F1 Pups (PND0) / males

Group		Pup weight (g)	Cube root of pup weight (g)	AGD of pups (mm)	Relative AGD of pups (mm)	Pup Nipple retention (N) PND 12
C 0 mg/kg bw per day	Mean	6.61	1.87	2.84	1.51	0.23
	SD	0.76	0.07	0.46	0.22	0.63
	N	142	142	142	142	117
LD 100 mg/kg bw per day	Mean	6.66	1.88	2.78	1.48	0.35
	SD	0.96	0.1	0.37	0.17	0.86
	N	123	123	123	123	98
MD 300 mg/kg bw per day	Mean	6.56	1.87	2.73	1.46	0.21
	SD	0.54	0.05	0.39	0.20	0.75
	N	137	137	137	137	117
HD 1000 mg/kg bw per day	Mean	6.42*	1.86	2.71*	1.46	0.04*
	SD	0.53	0.05	0.37	0.19	0.19
	N	159	159	159	159	134

Asterisks indicate statistically significant differences to control group C, *p < 0.05, **p < 0.01 and ***p < 0.001

Table 2 Summary AGD – F1 Pups (PND0) / females

Group		Pup weight (g)	Cube root of pup weight (g)	AGD of pups (mm)	Relative AGD of pups (mm)
C 0 mg/kg bw per day	Mean	6.29	1.84	1.26	0.68
	SD	0.69	0.07	0.25	0.14
	N	132	132	132	132
LD 100 mg/kg bw per day	Mean	6.23	1.84	1.15***	0.62***
	SD	0.69	0.07	0.20	0.10
	N	126	126	126	126
MD 300 mg/kg bw per day	Mean	6.24	1.84	1.13***	0.61***
	SD	0.59	0.06	0.29	0.16
	N	109	109	109	109
HD 1000 mg/kg bw per day	Mean	6.14	1.83	1.12***	0.61***
	SD	0.54	0.05	0.20	0.11
	N	149	149	149	149

Asterisks indicate statistically significant differences to control group C, *p < 0.05, **p < 0.01 and ***p < 0.001

Summary Anogenital Distance and Nipple Retention – F2 Generation, Cohort 1B

Table 3 Summary AGD F2, Cohort 1B / males

Group		Pup weight (g)	Cube root of pup weight (g)	AGD of pups (mm)	Relative AGD of pups (mm)	Pup Nipple retention (N) PND 12
C 0 mg/kg bw per day	Mean	6.39	1.85	2.98	1.61	0.33
	SD	0.57	0.06	0.41	0.20	0.73
	N	96	96	96	96	86
LD 100 mg/kg bw per day	Mean	6.51	1.87	2.89	1.55	0.2
	SD	0.64	0.06	0.35	0.18	0.52
	N	105	105	105	105	93
MD 300 mg/kg bw per day	Mean	6.39	1.85	2.87	1.55	0.42
	SD	0.64	0.06	0.31	0.16	0.82
	N	87	87	87	87	77
HD 1000 mg/kg bw per day	Mean	6.09**	1.82***	2.77***	1.52**	0.68**
	SD	0.61	0.06	0.34	0.18	0.93
	N	111	111	111	111	98

Asterisks indicate statistically significant differences to control group C, *p < 0.05, **p < 0.01 and ***p < 0.001

Table 4 Summary AGD F2, Cohort 1B / females

Group		Pup weight (g)	Cube root of pup weight (g)	AGD of pups (mm)	Relative AGD of pups (mm)
C 0 mg/kg bw per day	Mean	6.10	1.82	1.05	0.58
	SD	0.70	0.07	0.47	0.26
	N	79	79	79	79
LD 100 mg/kg bw per day	Mean	6.17	1.83	1.01	0.55
	SD	0.68	0.07	0.40	0.22
	N	107	107	107	107
MD 300 mg/kg bw per day	Mean	6.24	1.84	1.00	0.54
	SD	0.70	0.07	0.43	0.23
	N	103	103	103	103
HD 1000 mg/kg bw per day	Mean	5.99	1.81	1.06	0.59
	SD	0.63	0.06	0.39	0.21
	N	87	87	87	87

Asterisks indicate statistically significant differences to control group C, *p < 0.05, **p < 0.01 and ***p < 0.001

Historical data on AGD – Historical Control Data for Reproductive and Developmental Toxicity Studies

In the following historical control data on male and female AGD using Wistar rats are presented. These data are collected from more than 2000 Wistar rats from Reproductive and Developmental Toxicity Studies in the time period between 2016 and 2020 provided by BSL BIOSERVICE Scientific Laboratories Munich GmbH.

Table 5 AGD – Male Pups (PND0) – Historical control Data using Wistar Rat Year 2010-2017 / 2016-2020/ 2019-2020 - BSL BIOSERVICE

	Pup weight on PND 0 (g)	Cube root of pup weight	AGD (mm) of pups	Relative AGD of pups
Mean	6.5	1.9	2.6	1.4
SD	0.7	0.1	0.4	0.2
N	2073	2073	2073	2073
Mean – 2SD	5.05	1.72	1.81	0.99
Mean + 2 SD	7.88	2.00	3.48	1.85
Minimum	3.70	1.55	0.96	0.53
Maximum	8.80	2.20	4.21	2.18

Table 6 AGD – Historical control Data Female Pups (PND0) - using Wistar Rat Year 2010-2017 / 2016-2020/ 2019-2020 - BSL BIOSERVICE

	Pup weight on PND 0 (g)	Cube root of pup weight	AGD (mm) of pups	Relative AGD of pups
Mean	6.61	1.8	0.9	0.5
SD	0.7	0.1	0.3	0.2
N	2021	2021	2021	2021
Mean – 2SD	4.78	1.69	0.26	0.14
Mean + 2 SD	7.48	1.96	1.56	0.58
Minimum	3.10	1.46	0.27	0.16
Maximum	8.20	2.02	2.65	1.43

The comparison of male and female AGD in the study under discussion with historical control data clearly shows that the values are well within historical control values, even or especially after exposure to propyl 4-hydroxybenzoate up to the limit dose of 1000 mg/kg bw per day.

Although this comprehensive historical control data set for EOGRTSs from the CRO who has performed the key study central to the CLP proposal was provided on request to the evaluating MS Belgium, this data was apparently not considered when assessing and interpreting the findings leading to the C&L proposal. We consider this a significant shortcoming which adds to an apparent misrepresentative analysis and reporting of what should be objectively and reasonably concluded from the overall toxicological database for propyl 4-hydroxybenzoate.

2.2.2. Potential effect on Post implantation loss

The CLH-Dossier submitter raised a concern regarding developmental toxicity based on an apparent minor increased postimplantation loss without any statistically significant from the EOGRTS. Although in the F0

generation of the EOGRTS (Registration dossier (study report, 2021), the percentage of post implantation loss was slightly increased at the highest dose, this modification however was not dose-related (5.99, 7.79, 4.76, 8.98) and without statistical significance. More importantly, this effect was not confirmed in the cohort 1B animals (F1 generation) revealing that this finding is not test item related. As all the values are well within the range of the historical control data this observation is due to normal biological variation (Appendix II).

Appendix II- Data and discussion regarding post implantation loss based on EOGRTS

Table 7 Summary Parental Litter Data – Corpora Lutea and Implantation Sites

Group		Corpora Lutea (CL)	Implantation sites (IS)	Alive Pups on PND 0	Pre Implantation Loss (%)	Post Implantation Loss (%)
C 0 mg/kg bw per day	Mean	11.62	11.12	10.50	4.88	5.99
	SD	2.58	2.75	2.80	9.99	8.26
	N	26	26	26	26	26
LD 100 mg/kg bw per day	Mean	12.59	12.14	11.18	3.30	7.79
	SD	1.79	1.83	2.11	8.92	11.13
	N	22	22	22	22	22
MD 300 mg/kg bw per day	Mean	12.35	11.70	10.70	5.42	4.76
	SD	1.97	2.30	3.23	11.09	6.56
	N	23	23	23	23	22
HD 1000 mg/kg bw per day	Mean	12.04	11.67	10.89	3.20	8.98
	SD	2.03	2.02	2.66	6.88	19.18
	N	27	27	28	27	27

Table 8 Summary Parental Litter Data – Corpora Lutea and Implantation Sites- F1 Generation, Cohort 1B

Group		Corpora Lutea (CL)	Implantation sites (IS)	Alive Pups on PND 0	Pre Implantation Loss (%)	Post Implantation Loss (%)
C 0 mg/kg bw per day	Mean	10.37	10.32	9.26	0.38	9.05
	SD	3.09	3.04	3.77	1.64	22.93
	N	19	19	19	19	19
LD 100 mg/kg bw per day	Mean	11.55	11.05	10.60	3.45	4.11
	SD	2.78	2.56	2.68	9.57	7.06
	N	20	20	20	20	20
MD 300 mg/kg bw per day	Mean	12.17	10.94	10.56	8.90	4.90
	SD	2.60	2.62	2.96	17.63	10.77
	N	18	18	18	18	18
HD 1000 mg/kg bw per day	Mean	12.42	12.11	11.16	2.50	7.61
	SD	1.84	2.02	3.45	7.33	22.89
	N	19	19	19	19	19

Table 9 Historical control Data for Reproductive and Developmental Toxicity Studies - Using Wistar Rat Year 2010-2017 / 2016-2020/ 2019-2020 - BSL BIOSERVICE

	% pre-Implantation Loss	% Post-Implantation Loss
Mean	11.8	10.1
SD	16.2	20.08
N	508	507
Mean – 2SD	0.0*	0.0*
Mean + 2SD	44.2	51.8
Min	0.0	0.0
Max	85.7	100

The Comparison of post implantation loss with historical control data clearly show that all values are well within the range of the historical control data, even or especially after exposure to propyl 4-hydroxybenzoate up to the limit dose of 1000 mg/kg bw per day.

Although this comprehensive historical control data set for EOGRTS studies from the CRO who has performed the key study central to the CLP proposal was provided on request to the evaluating MS Belgium, this data was apparently not considered when assessing and interpreting the findings leading to the C&L proposal. We consider this a significant shortcoming which adds to an apparent misrepresentative analysis and reporting of what should be objectively and reasonably concluded from the overall toxicological database for propyl 4-hydroxybenzoate.

The CLD-dossier submitter mentioned that in the dose range finding study for reproduction/developmental toxicity screening (Registration dossier (study report, 2018)), the post-implantation loss increased slightly, but in a dose- dependent manner (6.47, 6.74 and 8.72 %, respectively at 0, 500 and 1000 mg/kg bw per day). However these changes are all still well within historical control data and – moreover - due to the low number of animals in this screening study these data can not be used as a basis for decisions regarding classification. Additionally it has to be mentioned that other parameters relevant for the development of pups were not affected up to the limit dose of 1000 mg/kg by per day in all available OECD-TG and GLP conform studies (OECD 443, OECD 422 and OECD 414) i.e. pre-implantation loss, implantation sites, live pups on PND 0 and corpora lutea. This supports that the slight and not statistically significant increase in post implantation loss is not considered to be test item related but due to biological variability.

2.2.3. Potential effects on fertility

The CLH-Dossier submitter raised a concern regarding fertility based on some minor effects in sperm motility and morphology from the EOGRTS. However, Clariant Produkte (Deutschland) GmbH does not agree with the above position provided by the Belgium MS. Based on the data from an EOGRTS the administration of propyl 4-hydroxybenzoate up to the limit dose of 1000 mg/kg bw per day did not cause any adverse effect on sperm parameters (sperm count, sperm motility and sperm morphology). The reduction of sperm motility (72.67 % HD vs 77.05 % in control) and sperm morphology (tail only) (8.17 vs 2.96 % in control) at 1000 mg/kg bw per day which was addressed by the CLH-Dossier submitter are not statistically significant but within the range of historical control data. More importantly, when looking at individual F0 parental values, the reduction in sperm motility in the HD group is coming from one single animal showing a very low sperm motility value (animal no. 92; 0% motility, correlated with histopathological finding of aspermia). However, this is also the case for control animal no. 22 (10.5% motility, correlated with histopathological finding of tubular edema), strongly suggesting an inherent strain-specific background incidence of testicular findings in animals with very low or absent sperm motility. Such situations lead to large standard deviations and subtle differences between groups have thus to be interpreted carefully (for more details please refer to Appendix III). Additionally, although

functional parameters that are related to sperms (e.g. male fertility index, mating index etc.) were not affected in the very comprehensive data set on reproductive toxicity from different OECD and GLP conform studies (EOGRTS and OECD 422), again no explanation was given by the CLH-dossier submitter why these effects were considered as adverse. A detailed description of the results from the EOGRTS on sperm parameter and functional reproductiv were summerzied in Appendix III.

Furthemore the CLH-dossier submitter raised a concern regarding male fertility after propyl 4-hydroxybenzoate exposure based on a study published by Oishi et al. (2002) which investigated effects of propyl 4-hydroxybenzoate on the male reproductive system in Wistar rats. However, a thorough scientific review of the reported data demonstrate clear shortcomings of this non-GLP and non-OECD TG study which limits the usefulness of this data for evaluation purposes. In this 4-week repeat-dose study, 21 days old juvenile male Wistar rats (n= 8 per group) were exposed to doses of 0.01, 0.1 or 1% propyl 4-hydroxybenzoate (99% purity) in the diet which correspond approximately to 0, 12.4, 125 and 1290 mg/kg bw per day. Information on daily clinical signs were not available which is one of the limitations of this study as general and systemic toxicity could not be evaluated. There were no treatment-related effects on testes, epididymides, ventral prostates, seminal vesicles and preputial glands in any of the groups. At all three dosage levels, however, a decrease in cauda epididymal sperm reserve, sperm count, and daily sperm production was observed and from 125 mg/kg/day on, serum testosterone concentration was decreased (LOAEL: 12.4 mg/kg/day). However it has to be noted that this was a non-GLP, non-guideline study with small group size (n=8). There were a number of control values in parameters that were well outside of the normal range. The data were not consistent with literature data and data from other studies of Oishi for epididymal sperm counts (**39.8 million per gram tissue in control animals** (Food Chem Tox 42: 1845-9 Oishi) vs. **1080 million per gram tissue in control animals** (Oishi 2002)). In addition, a full study protocol and raw data are no longer available which makes it difficult to reevaluate these data from scientific point of view. The highest dose tested in this study exceed the maximal dose of 1000 mg/kg bw per day which requested in OECD studies. More importantly as daily clinical signs were not evaluated in this study it cannot be ruled out that the effects on spermatogenesis are only secondary effects due to systemic toxicity. Additionally, although the CLH-dossier submitter mentioned in their synopsis that body weight was not evaluated in this study,the data published by Oishi and co-worker however clearly showed a reduction in terminal body weight in the high dose group exceeding the limit dose of 1000 mg/kg bw per day which may be considered as indicator for unpalatability and stress which has an impact on spermatogenesis. Most importantly, all reported findings from Oishi and co-workers, i.e. decrease in cauda epididymal sperm reserve, sperm count, daily sperm production serum testosterone concentration. could not be repeated in a study by Gazin et al. 2013 as described below.

Based on the limitations of the Oishi study (e.g. non-GLP, non- OECD, limited group size, limited evaluation of systemic toxicity, short period of treatment, absence of dose-response for DSP) the study was scored as Klimisch 3 by Clariant Produkte Deutschland. No explanation is given by the evaluating MS why despite all the limitations this study was used as a “key study” for deriving the CLH-proposal although more reliable studies having a higher Klimisch score are available.

In order to verify or further characterize the effects reported by Oishi 2002, additional GLP-compliant in vivo studies on the potential reproductive toxicity of propyl 4-hydroxybenzoate were conducted in male juvenile Wistar rats starting at PND 21 (Ricerca Biosciences 2012d). The findings of these studies were published by Gazin et al (2013) and reviewed by SCCS (2013) and EMA (2015). The main study (Ricerca Biosciences, 2012d) was conducted under GLP in general compliance with FDA (2006) and EMA (2008) guidelines on reproductive toxicity testing. The objectives of the main reproductive toxicity study (Ricerca Biosciences, 2012d) were to determine the toxicity of the test item propyl 4-hydroxybenzoate following daily oral administration to juvenile male Wistar rats from the age of weaning on post-natal day (PND) 21 through sexual maturation and up to 11 weeks of age (8-week treatment period). The selected treatment period covers the juvenile (PND 21-35), peri-pubertal (PND 35-55), pubertal (55-70) and early adult stages in the male rat. As in the Oishi (2002) study, the study was performed in the same strain of juvenile male rat (Wistar Crj: WI (Han) and treatment started on PND 21. However, the duration of exposure was extended from 4 to 8 weeks (PND 77) and gavage (once daily) was used instead of dietary administration. Furthermore, a fourth dose level-group (low dose) was included in an attempt to determine a NOAEL. Additional animals were included to evaluate the reversibility of any toxic signs during a 26-week treatment-free period (to cover 3 spermatogenic cycles). Additional endpoints such as histopathology and serum LH and FSH levels were included in order to determine the mechanisms of the awaited testicular and epididymal effects. The pathology data and evaluation were subjected to an external review. The vehicle was 1 % (w/v) hydroxyethylcellulose 80-125 centipoises at 2 % in water for injection. Purity of the test substance, stability in the vehicle and homogeneity of the test suspension were controlled. The test item was applied once daily by gavage and Group 1 animals (controls) received the vehicle alone. For the analysis of testosterone, LH and FSH, blood samples of about 2 ml were taken from the retro-orbital sinus of all animals under isoflurane anaesthesia from the animals fasted for at least 14 hours in the morning of PND 78 and PND 79.

The main results of the study are as follow (for more details see Appendix IV):

- No unscheduled deaths were observed.
- There was no influence of treatment on organ weight, mean body weight and BW gain in any group.
- There was no influence of treatment on time of sexual maturation of the males in any group.
- No influence of treatment on the levels of the measured hormones (LH, FSH and testosterone) was observed in any group.
- There were no effects of treatment on mean sperm counts and motility parameters at terminal sacrifice and sacrifice after the treatment-free period.
- Pathology investigations, daily oral administration of propyl 4-hydroxybenzoate in post-weaning juvenile male Wistar rats did not result in test item-related macroscopic or microscopic changes in the testes and epididymides.
- There was no evidence of any treatment-related effect on testicular and epididymal weights or on sperm count and motility data in any of the treated groups.

In conclusion, the NOAEL of the study for male reproductive endpoints is 1000 mg/kg bw per day for the treatment period of 8 weeks. The present study did not confirm the effects on the reproductive functions reported by Oishi et al. (2002) and is regarded of sufficient quality to overturn the findings by Oishi, which is a study that suffers from numerous limitations as mentioned above.

Appendix III - Details and discussion on Sperm parameter based on EOGRTS

In the EOGRTS at terminal sacrifice, left epididymis, left testis and left vas deferens were separated and used for evaluation of sperm parameters (Motility, testicular sperm head count and morphology) from all parental generation males and all cohort 1A males of each group were performed by using Hamilton Thorn Sperm Analyser (TOXIVOS Version 13.0C). Sperm morphology was performed manually by manual method. Sperm morphology slides were prepared from all parental generation and all cohort 1A males. Initially, evaluation was made in male animals of the groups 1 and 4 sacrificed at the end of the treatment period. Sperm morphology examinations were not extended to male animals of all other dosage groups as no treatment-related changes were observed in the high dose group. For morphology evaluation, sperm from left vas deferens were transferred to 0.1 % bovine serum albumin solution. For staining two drops of 1 % aqueous Eosin-Y solution was mixed with six drops of the sperm-suspension. The stained sperm suspension was used to prepare smears on slides. After complete drying, the slides were dipped into 0.1 % acetic acid for approximately 30 seconds to intensify the colouring.

Sperm Morphology:

Parental generation and F1-1A cohort males:

Evaluation of sperm morphology from control and HD parental and cohort 1A males did not show any indications for toxicity induced by the test item, and percentage of normal and abnormal sperms in treatment groups were comparable with the controls. The slight reduction in sperm morphology in Parentla generation

(95.88%→93.33) and in F1 generation – cohort 1A (94.83% → 90.74) was not statistically significant and within the range of hoistorical data which were provided by the CRO BSL (Table 12).

Table 10 Summary Sperm Morphology – parental males

		Head and Neck						
		Amorphous Head	Banana Shape Head	Hookless sperm	Pin head	Head only	Small head	Bent Neck
C	Mean	0 0	0 0	0 0	0 0	2 46	0 0	0 0
	SD	0 0	0 0	0 0	0 0	1 89	0 0	0 0
	N	24	24	24	24	24	24	24
HD	Mean	0 0	0 0	0 0	0 0	2 63	0 0	0 0
	SD	0 0	0 0	0 0	0 0	3 35	0 0	0 0
	N	24	24	24	24	24	24	24

		Tail				
		Bent Tail	Brocken Tail	Coiled Tail	Tail Only	Short
C	Mean	2 17	0 42	0 25	2 96	0 0
	SD	1 69	0 65	0 53	1 81	0 0
	N	24	24	24	24	24
HD	Mean	2 38	0 08	0 08	8 17	0 0
	SD	1 47	0 28	0 28	26 21	0 0
	N	24	24	24	24	24

		Number of sperms evaluated	Total Number of abnormal sperm/ findings	Total number of normal sperms/findings	% normal	% abnormal
C	Mean	200	191 75	8 25	95 88	4 13
	SD	0 0	3 53	3 53	1 76	1 76
	N	24	24	24	24	24
HD	Mean	200	186 67	13 33	93 33	6 67
	SD	0 0	28 83	28 83	14 42	14 42
	N	24	24	24	24	24

When looking at individual F0 parental values, a single HD group animal can be identified with a higher increased numbers of “Tail only” finding, as mentioned in the CLP report. This finding occurred 116 times in this animal whereas the remaining 17 animals of this group showed a total of 85 counts. Thus, like in the case of sperm motility, the higher mean of “Tail only” finding relies on a single animal that – as stated above – can potentially be regarded as background incidence. Control animal no. 22 was not evaluated for sperm morphology. Regardless of sperm motility and morphology findings, animal no. 92 was mated with female no. 202 which was sperm positive on the 5th mating day and gave birth to a litter of 11 alive pups.

Table 11 Summary Sperm Morphology – F1 generation, Cohort 1A

		Head and Neck						
		Amorphous Head	Banana Shape Head	Hookless sperm	Pin head	Head only	Small head	Bent Neck
C	Mean	0 0	0 0	0 0	0 0	3 9	0 0	0 0
	SD	0 0	0 0	0 0	0 0	1 86	0 0	0 0
	N	20	20	20	20	20	20	20
HD	Mean	0 0	0 0	0 0	0 0	5 0	0 0	0 0
	SD	0 0	0 0	0 0	0 0	4 26	0 0	0 0
	N	18	18	18	18	18	18	18

		Tail				
		Bent Tail	Brocken Tail	Coiled Tail	Tail Only	Short
C	Mean	2.35	0.2	0.05	3.85	0.0
	SD	1.57	0.41	0.22	1.69	0.0
	N	20	20	20	20	20
HD	Mean	2.06	0.67	0.17	11.17	0.0
	SD	1.70	1.14	0.51	26.33	0.0
	N	18	18	18	18	18

		Number of sperms evaluated	Total Number of abnormal sperm/ findings	Total number of normal sperms/findings	% normal	% abnormal
C	Mean	200.00	189.65	10.35	94.83	5.18
	SD	0.00	3.72	3.72	1.86	1.86
	N	20	20	20	20	20
HD	Mean	200.00	180.94	19.06	90.47	9.53
	SD	0	29.16	29.16	14.58	14.58
	N	18	18	18	18	18

The higher percentage of abnormal sperm in the F1 cohort 1A cohort (HD group 9.53% vs. C group 5.18%) is slightly above the historical control data range (Mean 4.59% with a 2SD range between 0.00% days and 9.50%). This is related to animal no. 298 showing 67% abnormal sperms. This animal also showed markedly low percentage of motile sperm, markedly high percentage of static sperm, markedly low percentage of rapid sperm, a low testis weight and histopathologically aspermia in the epididymides and tubular degeneration in the testes. When not considering HD animal no. 298, the rate of abnormal sperms in the HD did not considerably differ from Control group (HD 5.81% vs. C 5.18%) suggesting that this is possibly an incidental finding.

Table 12 Historical data on sperm Morphology - Using Wistar Rat Year 2010-2017 / 2016-2020/ 2019-2020- BSL BIOSERVICE

	Number of sperms evaluated	Total Number of abnormal sperm/ findings	Total number of normal sperms/findings	% normal	% abnormal
Mean	200.73	9.24	191.48	96.41	4.59
SD	2.97	5.03	5.03	2.45	2.45
N	99	99	99	99	99
Mean - 2SD	194.78	0.00	181.43	90.50	0.00
Mean + 2SD	206.67	19.3	201.45	100.31	9.50
Min	200	1	172	81.9	0.5
Max	220	38	208	99.5	18.1

Sperm Motility:

Based on data generated from the EORTS propyl 4-hydroxybenzoate had no effect on epididymal sperm motility count analysed from all males at terminal sacrifice by using Hamilton Thorn Sperm Analyser (TOX IVOS Version 13.0C). Although group mean motility values from parental and cohort 1A males were marginally lower in HD group, this effect was attributed to very low values from only one parental and cohort 1A male and sperm motility values from all other males were comparable with control and therefore this marginal decrease in group mean motility in the HD group was not considered as adverse. More importantly it

has to be mentioned that these effects were without statistically significance, non dose dependent and in range of historical control data as show in Table 15.

Table 8 Summary Motility – Parental Males

Group		Motile Count [%]	Static Count [%]	Rapid [%]
C 0 mg/kg bw per day	Mean	77.05	22.97	60.85
	SD	13.45	13.44	12.38
	N	30	30	30
LD 100 mg/kg bw per day	Mean	77.60	22.04	57.34
	SD	26.71	5.36	9.69
	N	30	25	25
MD 300 mg/kg bw per day	Mean	77.98	22.02	60.68
	SD	3.59	3.59	7.28
	N	25	25	25
HD 1000 mg/kg bw per day	Mean	72.67	24.00	55.75
	SD	19.50	14.58	16.10
	N	30	30	30

Table 9 Summary Sperm Motility – Cohort 1A Males

Group		Motile Count [%]	Static Count [%]	Rapid [%]
C 0 mg/kg bw per day	Mean	79.10	20.90	64.83
	SD	6.76	6.76	8.22
	N	20	20	20
LD 100 mg/kg bw per day	Mean	78.33	21.68	62.88
	SD	7.82	7.82	10.90
	N	20	20	20
MD 300 mg/kg bw per day	Mean	78.87	21.13	62.13
	SD	4.82	4.82	8.14
	N	19	19	19
HD 1000 mg/kg bw per day	Mean	72.42	27.58	58.11
	SD	21.67	21.67	19.77
	N	18	18	18

Table 10 Historical control data on sperm motility - Using Wistar Rat Year 2010-2017 / 2016-2020/ 2019-2020- BSL BIOSERVICE

	Motile Count [%] (mean)	Static Count [%] (mean)	Rapid [%] (mean)
Mean	81.71	18.29	65.25
SD	8.23	8.23	11.31
N	114	114	114
Mean - 2SD	65.25	1.83	42.62
Mean + 2SD	98.17	34.75	87.88
Min	44.5	4.5	17.0
Max	95.5	55.5	79.5

When looking at individual F0 parental values, a single HD group animal can be identified with a very low motility value (animal no. 92; 0% motility, correlated with histopathological finding of aspermia). However, this is also the case for control animal no. 22 (10.5% motility, correlated with histopathological finding of tubular edema), strongly suggesting an inherent strain-specific background incidence of testicular findings in animals with very low or absent sperm motility. These lead to large standard deviations and subtle differences between groups have to be interpreted carefully.

Moreover, sperm motility results were within the range of the lab's historical control data (mean: 81.71% with a \pm SD range between 73.48 and 89.94%). Additionally, it should be noted that all other groups, including Controls were also on the lower side of the historical control data range.

In conclusion it should be added that sperm motility was not statistically significantly different between dose groups and control group, neither in F0 parental nor in F1 cohort 1A.

Testicular Sperm Head Count:

Parental generation and F1-1A cohort males:

Test item had no effect on sperm head count in the dose groups of this study. No considerable and statistically significant differences were observed between dose and control groups of parental animals and animals of the cohort 1A.

Table 16 Mean Testicular Sperm Count - F0 Parental

Group		Million Sperms/g
C 0 mg/kg bw per day	Mean	113.5
	SD	22.7
	N	30
LD 100 mg/kg bw per day	Mean	115.5
	SD	31.3
	N	25
MD 300 mg/kg bw per day	Mean	124.0
	SD	25.8
	N	25
HD 1000 mg/kg bw per day	Mean	114.9
	SD	28.8
	N	30

Table 11 Mean Testicular Sperm Count - F1 Generation, Cohort 1A

Group		Million Sperms/g
C 0 mg/kg bw per day	Mean	127.6
	SD	33.0
	N	18
LD 100 mg/kg bw per day	Mean	126.8
	SD	28.8
	N	19
MD 300 mg/kg bw per day	Mean	131.5
	SD	22.4
	N	18
HD 1000 mg/kg bw per day	Mean	137.2
	SD	31.9
	N	16

Table 12 Historical control Data for Reproductive and Developmental Toxicity Studies - Wistar Rat Year 2010-2017 / 2016-2020/ 2019-2020

	Million Sperms/g (mean)
Mean	114.45
SD	28.51
N	123

Mean – 2SD	57.42
Mean + 2SD	171.47
Min	28
Max	196.8

The evaluation of sperm parameters in the EOGRTS clearly indicate that no significant effects were observed in Sperm morphology, sperm motility as well as sperm count. All the values were within historical control data (Table 12 and 15). Additionally, it should be noted that neither in the F0 parental nor in F1 cohort 1A were there any indications showing that a tendency towards lower sperm motility or increased morphological findings are associated with an effect on fertility and development of the respective next generation. This refers to reproductive indices as percent male mating index, female mating index, male fertility index, female fertility index, gestation index (Table 19 and 20).

Therefore, the tendency towards lower sperm motility and the sperm morphological finding of “tail only” is not assumed to be a clear toxic sign with respect to male fertility and further investigation is considered an appropriate measure in order to clarify the situation.

Functional parameters related to reproduction and fertility based on EOGRTS

Table 19 Reproductive Indices in parental generation- OECD 443

Group		Male mating index (%)	Female mating index (%)	Male fertility index (%)	Female fertility index (%)	Gestation Index (%)
C 0 mg/kg bw per day	Mean	86.67	100.00	86.67	100.00	100.00
LD 100 mg/kg bw per day	Mean	92.00	100.00	92.00	100.00	100.00
MD 300 mg/kg bw per day	Mean	92.00	100.00	92.00	100.00	95.00
HD 1000 mg/kg bw per day	Mean	93.33	96.67	93.33	100.00	96.43

Table 20 Reproductive Indices in cohort 1B - OECD 443

Group		Male mating index (%)	Female mating index (%)	Male fertility index (%)	Female fertility index (%)	Gestation Index (%)
C 0 mg/kg bw per day	Mean	100.00	100.00	100.00	100.00	94.73
LD 100 mg/kg bw per day	Mean	100.00	100.00	100.00	100.00	100.00
MD 300 mg/kg bw per day	Mean	90.00	90.00	90.00	90.00	100.00
HD 1000 mg/kg bw per day	Mean	95.00	95.00	95.00	95.00	94.73

2.2.4. Summary of adverse effect on fertility and sperm parameter

To evaluate the effects of propyl 4-hydroxybenzoate on male fertility all available data with adequate quality (GLP and OECD) need to be considered (according to CLP 3.7.2.3. weight of evidence). Considering the CLH report provided by the MS Belgium it is our concern that a scientifically, and via CLP regulation required, balanced, transparent and objective assessment of all available data has not been carried out. Instead, it appears that negative data were not given equal weight with more weight being given to seemingly positive outcomes. To illustrate this in the following table the values highlighted in black were considered in the CLH-dossier as relevant for classification whereas the values presented in red were not taken into account for the analysis.

Table 21 Summary table of effects on male reproductive system

Dose (mg/kg bw per day)		0	3	10 - 12.4	100	125	300	1000	1290	Conclusion		
Sperm parameter												
Sperm count	Registration dossier (study report, 2021)-EOGRTS-OECD 443	Million Sperms/g	P	113 5	-	-	115 5	-	124 0	114 9	-	No effect observed
			Fl-CIA	127 6	-	-	126 8	-	131 5	137 2	-	
	Oishi et al, 2002	epididymal sperm conc. x10 ⁷ /g)		108	-	70.8	-	63.1*	-	-	48.8*	Non-GLP and non OECD (Klimisch 3) systemic toxicity cannot be ruled out, small group size, Highest dose exceed the limit dose of 1000 mg/kg according to OECD
	Gazin et al., 2013	Million sperm/g testis (SD)		110 3 (13 4)	103 1 (20 8)	106 0 (17 9)	111 2 (19 4)	-	-	105 4 (19 1)f	-	No effect observed (no significance and no dose dependency)
Epididymal sperm count Number of sperm			428 ± 202	501 ± 204	449 ± 271	473 ± 212	-	-	547 ± 198	-	No effect observed	
Sperm morphology	Registration dossier (study report, 2021)-EOGRTS-OECD 443	% normal sperm	P	95.88	-	-	-	-	93.33	-	Within historical contro- effects statistically NOT significant, function parameter were not affected (fertility index)	
			Fl-CIA	94.83	-	-	-	-	90.47	-		
Sperm motility	Registration dossier (study report, 2021)-EOGRTS-OECD 443	Motile Count [%]	P	77.05	-	-	77.60	-	77.98	72.67	-	Within historical control effects statistically NOT significant
			Fl-CIA	79.10	-	-	78.33	-	78.87	72.42	-	
	Gazin et al., 2013	Motile sperm ratio (%)		81 1 ± 12 5	88 2 ± 5 4	71 4 ± 28 8	-	-	85 5 ± 9 5	85 8 ± 9 6	-	No effect observed
Testis weight (in g or %)												
Registration dossier	P	Abs	1 91	-	-	1 903	-	1 95	1 93	-		
		Rel	0 42	-	-	0 42	-	0 44	0 43	-		

(study report, 2021)- EOGRTS- OECD 443	testis left										Within historical control- effects statistically NOT significant , no dose dependency
	P	Abs	1 90	-	-	1 88	-	1 92	1 89	-	
		Rel	0 42	-	-	0 41	-	0 43	0 42	-	
	testis right	Abs	1 73	-	-	1 76	-	1 75	1 65	-	
		Rel	0 50	-	-	0 50	-	0 52	0 51	-	
	F1- CIA (testis left)	Abs	1 70	-	-	1 69	-	1 71	1 60	-	
		Rel	0 49	-	-	0 48	-	0 51	0 49	-	
	F1 - CIA testis right	Abs	1 84	-	-	1 88	-	1 86	1 92	-	
Rel		0 45	-	-	0 46	-	0 45	0 47	-		
F1- C1B testis left	Abs	1 83	-	-	1 86	-	1 88	1 92	-		
	Rel	0 45	-	-	0 46	-	0 45	0 47	-		
F1-C1B testis right	Abs	1 83	-	-	1 86	-	1 88	1 92	-		
	Rel	0 45	-	-	0 46	-	0 45	0 47	-		
Oishi et al., 2002		Abs	2 65	-	2 67	-	2 60	-	2 60	No effect observed	
		Rel	0 96	-	0 95	-	0 95	-	0 99		
Registration dossier (study report, 2019)- 90 day study- OECD 408		abs	3 61	-	-	3 69	-	3 70	3 74	No effect observed	
		Rel	0 93	-	-	0 95	-	0 94	0 97		
Registration dossier (study report, 2012)- OECD 422	Testis left	abs	2 05	-	-	2 09	-	2 15	2 09	No effect observed effects statistically NOT significant ,No dose dependency, within historical control	
		Rel	0 49	-	-	0 49	-	0 50	0 50		
	Testis right	Abs	2 03	-	-	2 04	-	2 10	2 07		
		Rel	0 48	-	-	0 48	-	0 49	0 50		
Gazin et al., 2013	Testis left g (SD)	Abs	1 93 (0 15)	2 03 (0 22)	1 96 (0 24)	2 11 (0 22)	-	-	2 14 (0 09)	No effect observed	
Prostate weight (in mg or %)											
Registration dossier (study report, 2021)- EOGRTS – OECD 443	Parenta l	Abs	3.28	-	-	3.22	-	3.006	2.856**	-	Within historical control data, no dose dependency (in F1), effects were not revealed in F1 generation
		Rel	0.72	-	-	0.71	-	0.67	0.64*	-	
	F1 CIA	Abs	1 842	-	-	1 74	-	1 89	1 72	-	
		Rel	0 53	-	-	0 49	-	0 56	0 53	-	
	F1 C1B	Abs	2 47	-	-	2 65	-	2 64	2 65	-	
		Rel	0 60	-	-	0 65	-	0 64	0 65	-	
Registration dossier (study report, 2019)- 90 day study- OECD 408		Abs	2 67	-	-	2 79	-	2 70	2 65	No effect observed	
		Rel	0 69	-	-	0 71	-	0 68	0 60		
Histopathology of male reproductive organs											
Registration dossier (study report, 2012)		NE	-	-	-	NE	-	NE	NE	-	No effect observed
Gazin et al., 2013		No notewor thy findings	Minimal focal or multifoc al tubular atrophy/ hypopla sia was recorded in the	No notewor thy findings	No notewor thy findings	-	-	Minimal focal or multifoc al tubular atrophy/ hypopla sia was recorded in the	-	No effect observed	

			testis of 3/10 animals					testis of 1/10 animals		
Registration Dossier (study report, 2021) - EOGRTS- OECD 443	P	NAE	-	-	NAE	-	NAE	NAE	-	No effect observed
	F1	NAE	-	-	NAE	-	NAE	NAE	-	
Testosterone conc. (ng/ml)										
Gazin et al., 2013		16.9 (13.9) ^c	17.6 (10.5)	21.2 (11.9)	22.9 (14.0)	-	-	18.9 (11.1) ^e		No effect observed
Oishi et al., 2002		9.08	-	8.20	-	7.17	-	-	5.86 [*]	non-GLP and non OECD (Klimisch 3) systemic toxicity cannot be ruled out, small group size, Highest dose exceed the limit dose of 1000 mg/kg according to OECD

Abs: Absolute; NAE: no adverse effect; Rel: relative

3. Overall conclusion on developmental and reproductive toxicity

In order to evaluate the effect of propyl 4-hydroxybenzoate on reproductive and developmental toxicity all available data with adequate quality must be considered. In this regard the extended one-generation reproductive toxicity study (OECD TG 443) represents the most sensitive, most robust and most comprehensive study for detecting DART effects that may occur as a result of pre- and postnatal substance exposure. This study provides information on gonadal function, the oestrus cycle, epididymal sperm maturation, mating, conception, gestation, parturition, lactation, weaning, and growth and development of the offspring. To further detect adverse effects on the pregnant female and development of the embryo and fetus consequent to exposure of the female from implantation to the day prior to parturition the Prenatal development toxicity study (OECD 414) provides detailed information that need to be considered.

Based on generated data from the EOGRTS according to OECD TG 443 and the prenatal development toxicity study according OECD TG 414 no concern regarding fertility, development and reproductive endpoints could be detected as summarized in a peer reviewed overview on reproductive toxicity potential of propyl 4-hydroxybenzoate (Fayyaz et al, 2021). Together with all other relevant data on reproductive toxicity it therefore can be concluded that propyl 4-hydroxybenzoate causes no toxicologically relevant alterations in any of the parameters addressed in the OECD TG 443 and/or OECD TG 414 on reproductive and developmental endpoints up to the limit dose of 1000 mg/kg bw per day. This conclusion is supported by a recently finalized SCCS reevaluation (SCCS/1623/20) on propyl 4-hydroxybenzoate conducted according to the European Commissions ‘Call for data on ingredients with potential endocrine-disrupting properties used in cosmetic products’. For this reevaluation the original study report of the EOGRTS and OECD 414 were provided and considered by the SCCS in its opinion to conclude that the NOAEL for reproductive and developmental toxicity can be set as 1000 mg/kg bw per day.

As already mentioned beside the OECD conform studies there are other experimental studies with adequate quality on propyl 4-hydroxybenzoate available revealing the fact that the NOAEL for reproductive and

developmental toxicity is 1000 mg/kg bw per day (Fayyaz et al. 2021). All available *in vivo* animal reproductive studies that are available for propyl 4-hydroxybenzoate are summarized in Appendix IV.

As a general aspect Clariant Produkte (Deutschland) GmbH notes that the concerns raised by the CLH-dossier submitter are only based on minor changes for single endpoints. None of these effects were statistically significant and none of the effects did follow a dose dependent manner. More importantly, all values were within the range of historical control data which was, however, not considered by the evaluating member state. We consider this a significant shortcoming which adds to an apparent misrepresentative analysis, evaluation and reporting of what should be objectively and reasonably concluded from the overall toxicological database for propyl 4-hydroxybenzoate.

Appendix IV- *in vivo* data on propyl 4-hydroxybenzoate

Sivaraman et al (2018): This study was designed to meet the requirements of the European Medicines Agency (EMA), Committee for Human Medicinal Products (CHMP), “Guideline on the need for Nonclinical Testing in Juvenile Animals on Human Pharmaceuticals for Paediatric Indications”; the United States Food and Drug Administration (US FDA) Guidance Document, “Nonclinical Safety Evaluation of Pediatric Drug Products”; and the ICH S3a guidelines, “Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies”. The studies were also conducted in compliance with the OECD Principles of Good Laboratory Practice and the US Food and Drug Administration Good Laboratory Practice for Nonclinical Laboratory Studies, 21 CFR Part 58.

Two separate studies with propyl 4-hydroxybenzoate were conducted to assess the potential estrogen-mimetic effects on: (1) reproductive development and function in male and female rats when administered on PNDs 4 to 90; and (2) uterine weights in immature female rats when administered on PNDs 21 to 23.

The GLP-compliant juvenile rat reproductive development and function study was conducted in Charles River Laboratories Montreal ULC (CR MTL), Senneville, Quebec, Canada and sponsored by Bristol-Myers Squibb Company, New Brunswick, New Jersey, USA.

A total of 34 time-mated Crl:CD(SD) Sprague-Dawley females rats (F0 generation) were received from Charles River Canada Inc. (St. Constant, QC, Canada) on Gestation Day (GDs) 18 (GD 0=the day a vaginal plug was observed). These dams were used to produce the F1-generation litters and for the cross-fostering/nursing of litters. The F1 generation was the experimental population. On PND 3 (PND 0=the day all pups in a litter were delivered by the dam), the litters of the dams were culled to 8 pups each (4/sex/litter, where possible), then assigned to cross-fostered litters (4/sex/litter; where possible) using a randomization procedure such that no more than one sibling from a given sex was assigned to a cross-fostered litter and no pup was assigned to its biological mother. At the initiation of dosing on PND 4, the F1-generation pups weighed 8.5–13.6 g (males) and 8.7–13.6 g (females).

propyl 4-hydroxybenzoate was administered orally once daily to groups of Crl:CD(SD) rats at doses of 0 (vehicle), 10, 100, or 1,000 mg/kg on Postnatal Days (PNDs) 4–90.

Cage-side clinical observations were conducted before and after dosing. Body weights for all F1 generation pups were measured daily from PND 3–21, twice weekly from PND 22 to 56, and on the day of scheduled euthanasia (fasted). Body weights for pregnant F1 females were measured on GDs 0, 3, 9, 12, 15, 18, and 21. Pregnant naïve females used only for mating with treated F1 males were weighed on GDs 0, 6 and 13. The onset of sexual maturation was determined by evidence of vaginal patency in females or preputial separation in males. Estrous cycling for all F1 generation females (25/group) was determined by cell cytology in vaginal lavage collected daily during the final 14 days of treatment (PND 76–89) to assess any potential effects on cycling. All F1 males and females (including concurrent vehicle-treated controls) were cohabitated with treatment naïve sexually mature partners in a 1:1 ratio for a maximum of 14-days starting between PND 90–92. Each morning of the cohabitation period, the females were examined for evidence of copulation (i.e., observation of copulatory plug or presence of sperm in vaginal lavage). The day of positive identification of spermatozoa was designated as GD 0, after which males and females were separated and returned to individual housing. On GD 13, cesarean-section/uterine examinations of all (including concurrent vehicle-treated controls) untreated pregnant females included: numbers of corpora lutea; numbers and distribution of implantation sites; size, color, and shape of the placentae; numbers of live and dead embryos; and numbers of early resorptions. Treated females and their concurrent vehicle-treated controls were allowed to litter and examined 3 times a day for the onset/completion of parturition and pup fostering behaviors. The day of completion of littering is designated as lactation day (LD) 0 for the F1 dams and PND 0 for their offspring (F2), respectively. The F2-generation pups were evaluated for survival, sex, gross external dismorphology, and body weight and euthanized thereafter on PND 4–6. The F1 dams were terminated on LD 5–7.

Results:

No propyl 4-hydroxybenzoate -related clinical signs at 10 or 100 mg/kg/day were observed. At 1000 mg/kg/day, the incidence of animals with abdominal distention and salivation was increased, when compared to controls but this was considered due to an adaptive reflex to the large amount of test material administered. No propyl 4-hydroxybenzoate -related effects on the body weight at 10 or 100 mg/kg/day were observed. At 1,000 mg/kg/day, mean absolute body weights were slightly increased but this was related to slightly higher food consumption and was not considered treatment related. No propyl 4-hydroxybenzoate -related changes in the age of vaginal patency in females or preputial separation in males at any dose were observed. The mean age of onset of vaginal patency in females at 1,000 mg/kg/day was statistically significantly lower (accelerated) than controls (mean day of 31.2 days versus 33.9 days in controls); however, the value remained within the Test Facility's historical control range (ranging from 29.0 to 33.9 days) and published control range (28–41 days). The decrease in the current study appears to have been skewed by 7/25 control females with late development (35–43 days) resulting in a high control mean value (PND 33.9); notably, the control mean in this study is equal to the upper limit of the historical control range. Additionally, at 1,000 mg/kg/day, 3 pups with the lowest ages at vaginal opening (PND 28) were littermates. At 1,000 mg/kg/day, mean body weight on the day of vaginal opening was statistically significantly lower compared to control mean (115.2 g versus 128.6 g in controls); this was unexpected, since age upon landmark attainment is usually a function of weight, such that females who are smaller will generally demonstrate vaginal opening on later dates. As such, the lower age at

which vaginal patency was attained is considered spurious, and likely attributed to the larger mean control value. In routine body weight collections for the entire duration of this period (PND 28–35), weights were comparable across experimental groups. Therefore, neither finding (day of patency or body weight) is attributed to PP treatment, nor considered adverse. PP treatment had no impact on preputial separation in males.

Main observations: No propyl 4-hydroxybenzoate -related effects on estrous cyclicity. • No propyl 4-hydroxybenzoate -related effects on mating or fertility indices, mean number of days to mating, or the conception rate of the treated females paired with untreated males

Harlan (2012)(according to OECD 422): In a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test according to OECD 422 and GLP, propyl 4-hydroxybenzoate was administered to 11 rats per sex and group in doses of up to and including 980 and 1076 mg/kg bw per day in the diet to males and females. propyl 4-hydroxybenzoate was administered orally in the diet daily at concentrations of: 0, 1500, 4500 and 15000 ppm. These doses correspond to the actual doses in mg/kg/day as noted in Table 13 above. Test item was administered to male rats for 28 days and to female rats for 14 days prior to pairing, through the pairing and gestation periods until the F1 generation reached day 4 post partum.

No signs of adverse toxicity were observed in males or females at any dose level following repeat daily dosing. At the dose level of 15000 ppm in males, reduced body weight gain was noted in the absence of statistically significant changes in absolute body weights and an increase in triglycerides concentration was noted in the absence of any histopathological or other changes related to the finding. No further test item-related effects were noted in males or females at any dose level. Sperm motility, morphology and sperm count, estrus cycle, mating performance, fertility, duration of gestation, corpora lutea count, implantation rate, post implantation and postnatal loss and litter size were similar in the control and all dose groups. There were no test item-related findings in pups noted during the first litter check, the first 4 days post partum or during the necropsy. Based on the results of this study, the NOAEL for general toxicity and toxicity to reproduction (fertility) of propyl 4-hydroxybenzoate was considered to be 980 mg/kg bw per day for males and 1076 mg/kg bw per day for females.

EOGRTS dose-range finding study (similar to OECD 421) (2018): In a dose range finding study for according to OECD 421 propyl 4-hydroxybenzoate was administered daily in graduated doses to 2 groups of test animals, one dose level per group, 7 days per week. The aim of this screening study was to generate toxicological relevant information on possible effects on fertility and embryofetal development after repeated administration (gavage) in Wistar rats. Males were dosed until the minimum total dosing of 35 days, during 21

days of pre-mating and maximum 14 days of mating. All females were dosed during 21 days of pre-mating and up to 14 days of mating. One dam of each treatment group was dosed up to GD 20 (day of caesarian section). The other dams were dosed during gestation and up to PND 21. The pups of 3 litters (one from each group) were treated from PND 13 to PND 21, in accordance with the treatment group of the dam. The last administration of the test item formulations or vehicle was 30 minutes (+/- 10 minutes) before final necropsy. The study included 50 Wistar rats, 5 male and female animals in the control group, and 10 male and female animals in the test item group. Propyl 4-hydroxybenzoate was administered orally once daily at doses of 0, 500 and 1000 mg/kg bw per day.

The test item formulation was prepared freshly on each day of administration with 1 % aqueous hydroxyethyl-cellulose. Dose volumes were adjusted individually based on weekly body weight measurements. The administration volume was 5 mL/kg body weight. During the period of administration, the animals were observed each day for signs of toxicity. Body weight and food consumption were measured weekly, except for food consumption measurements which were not taken during the mating period in female animals and the mating and post-mating period in male animals.

After 21 days of treatment to both male and female, animals were mated (1:1, if possible) for a maximum of 14 days. The subsequent morning onwards the vaginal smears of females were checked to confirm the evidence of mating. After the confirmation of the mating, females were separated and housed individually. Each litter was examined as soon as possible after delivery of the dam to establish the number and sex of pups, stillbirths, live births, runts and the presence of gross abnormalities. Live pups were counted, sexed and litters weighed within 24 hours of parturition, on day 4 and day 13 post-partum. 1 female animal of each dosing group was sacrificed on the respective gestation day 20. Following the gross necropsy, the uteri and ovaries were removed, weighed and examined for number of implantations, resorptions (early and late), live and dead foetuses. Foetuses were identified by numbered strings, sexed and weighed. All foetuses were observed for external abnormalities. The males were sacrificed after completion of the mating period on treatment days 35 and the females along with their pups were sacrificed on post-natal day 21. The number of implantation sites and corpora lutea was recorded for each parental female at necropsy.

Pups sacrificed on post-natal day 4 or 13 and those found dead, were carefully examined for gross external abnormalities. All gross lesions macroscopically identified were examined microscopically in all animals.

At the conclusion of the test, surviving animals were sacrificed and observed macroscopically. Urine was collected at terminal sacrifice from C and HD female animals.

Treatment of the animals with propyl 4-hydroxybenzoate was not associated with test item-related morbidity and mortality. In the repeated dose part of the study, moving the bedding and increased salivation was noted on some days of treatment in 5/10 males of the HD group. These slight clinical signs were mostly observed directly after dose application or in anticipation thereof and thus were considered to be signs of discomfort due to oral gavage and/or a local reaction to the test item or vehicle rather than a systemic adverse effect and have no toxicological relevance. Food consumption and body weight development up to 1000 mg/kg bw per day were not affected by the treatment with propyl 4-hydroxybenzoate.

The copulation, fertility, delivery and viability indices were not affected in the dose groups and controls. There was no toxicologically relevant effect on length of pre-coital interval and duration of gestation.

There was no test item-related effect on pre implantation loss and post implantation loss, total amount of pups and sex-ratio. No runts were seen in any of the groups. There were no meaningful differences between the dose groups and controls for sex ratio of pups.

No test item related effect on mean mortality of pups between PND 0-4, PND 4-13 and PND 13-21 in treatment groups was observed when compared to the control group.

No remarkable, toxicologically relevant external gross abnormalities were observed in pups of any group.

No macroscopic finding was recorded after the treatment with propyl 4-hydroxybenzoate.

At histopathology, no test item related changes were observed in the examined brain samples at Hematoxylin & Eosin Stain, Fluoro-Jade® staining at immunohistochemistry with Glial fibrillary acidic protein (GFAP).

On the basis of this dose-range-finder test with propyl 4-hydroxybenzoate in male and female Wistar rats with dose levels of 500, and 1000 mg/kg body weight/ day the following conclusions can be made:

Repeated treatment with propyl 4-hydroxybenzoate was not associated with test item-related morbidity and mortality up to a dose level of 1000 mg/kg bw per day. There were no signs of general toxicity in the parental animals. There were no signs of reproductive or developmental toxicity in this study up to a dose level of 1000 mg/kg bw per day.

Prenatal Developmental Toxicity Study (2018) (according to OECD TG 414)- The aim of this study was to assess possible adverse effects on pregnant females and embryo-foetal development which could arise from repeated exposure of propyl 4-hydroxybenzoate via oral administration (gavage) to female rats during gestation days 5 to 19. Nulliparous and non-pregnant females were mated with males (2:1 ratio) and divided into four groups based on their body weights on the day of sperm positive vaginal smears (GD 0). The 4 groups comprised 25 female Wistar rats. Propyl 4-hydroxybenzoate was administered orally once daily at doses of 0, 100, 300 and 1000 mg/kg bw per day. During the period of administration, the animals were observed precisely each day for signs of toxicity and mortality. All female animals were sacrificed on the respective gestation day 20. Following the gross necropsy, the uteri and ovaries were removed, weighed and examined for number of implantations, resorptions (early and late) live and dead foetuses. The uteri of the non-pregnant females were processed with 10 % ammonium sulphide solution and checked for the early embryonic deaths. Body weight and food consumption were measured on gestations days 0, 5, 8, 11, 14, 17 and 20. Foetuses were identified by color strings, sexed and weighed. All foetuses were observed for external abnormalities. One half of each litter was examined for soft tissue anomalies by a microdissection technique. The remaining foetuses were processed by Alizarin red staining and the first 20 litters per group were examined for skeletal alterations. Craniofacial examination of the heads of the foetuses used for the soft tissue examination of at least 20 litters per group was performed for internal structure including the eyes, brain, nasal passage and tongue by razor blade serial sectioning technique.

Maternal Findings

No mortality was observed during the treatment period and all animals survived until end of the study.

In terminally sacrificed females, predominant clinical signs observed were moving the bedding (16/25 in HD), increased salivation (5/25 in HD) and piloerection (5/26 in Control and LD, 7/25 in MD and 12/25 in HD) were observed on few days during the treatment period of the study.

As moving the bedding and salivation was noted mainly immediately after test item administration and just for a short period, this transient sign was considered to be an unspecific sign due to a local reaction to the test item administration rather than a systemic adverse effect. Piloerection was observed on very few days and in all groups including control. Therefore, all clinical signs observed in terminally sacrificed treatment group females were of no toxicological relevance or non adverse in nature.

None of the females showed signs of abortion prior to the scheduled sacrifice.

The mean body weight and body weight gain remained unaffected throughout the study period. No statistical significance was achieved in any treatment groups on any day or interval of body weight measurement and all values in treatment groups were comparable with the controls.

In correlation to the body weight and body weight gain, food consumption in all treatment groups was comparable to the controls and no statistically significant effect was observed on food consumption in treatment groups on any interval of food consumption measurement when compared with the controls.

No test item-related effects of toxicological relevance were noted for any prenatal parameters like terminal body weight, uterus weight, adjusted maternal weight, number of corpora lutea, implantation sites, early and late resorptions, number of live foetuses, number of male and female foetuses, number of foetuses in each uterine horn, sex ratio, and percent pre- and post-implantation loss in treatment groups when compared to the controls. No dead foetuses were noted in any of the groups.

Successful mating resulted in 23/25 pregnancies in the LD group, 21/25 in the MD group and 24/25 in the HD group compared to 20/25 pregnancies in the control group. The marginally low pregnancy rates (no. of pregnancies / no. of females mated or sperm positive x 100) of 77 % in the control group compared to treatment groups (92 % in LD, 84 % in MD and 96 % in HD) was considered to be a biological variation.

No gross pathological changes of toxicological relevance were observed during the macroscopic examination of the females of the LD, MD and HD groups.

Foetal Findings

There were no test item-related effects of toxicological relevance observed for the mean foetus weight, total litter weight, male and female litter weight in any of the treatment groups when compared with the controls.

There were no external abnormalities considered to be of toxicological relevance in any of the dose groups. Statistical analysis of data revealed no significant differences compared to the control group.

Internal observation of the foetal viscera by free hand microdissection technique revealed a range of visceral findings in all groups including control. Visceral findings observed in the dose groups were at frequencies generally comparable to or in some cases slightly higher or lower in frequency compared to controls. As observed findings were either minor variations, within historical control data range and/or due to a lack of dose dependency and consistency, no toxicological significance can be attributed to these findings and they were considered to be spontaneous in nature.

Skeletal examination of the Alizarin red stained fetuses revealed a range of findings which occurred at an incidence generally comparable to or slightly lower or higher in the dose groups when compared to the control group.

There was no statistical significance and no indication of a test item-related trend in the type and/or incidences of other skeletal findings and they were therefore considered to be spontaneous in nature. On the basis of this prenatal developmental toxicity study in pregnant Wistar female rats with propyl 4-hydroxybenzoate at dose levels of 100, 300, and 1000 mg/kg body weight/ day administered on gestation days 5 to 19, the following conclusions can be made: No mortality was observed in the study and there were no clinical signs of toxicological relevance observed in the treatment groups. The body weight, food consumption, prenatal, litter data and gross pathology of terminally sacrificed females remained unaffected in the treatment groups when compared to the controls. Furthermore, no treatment-related and toxicologically relevant external, visceral or craniofacial findings were observed in the HD group and other treated groups. Findings of reduced ossification of some bones and few other skeletal findings were well within the historical control data range for this strain of rats and not considered to be a substance related effect. Generally delayed ossification is not regarded to persist postnatally and not associated with long-term consequences on survival, general growth and development and therefore is not considered to be adverse. No effects of propyl 4-hydroxybenzoate on females and fetuses were found at dose levels up to 1000 mg/kg body weight/day. The NOAEL for both maternal toxicity and foetal toxicity of propyl 4-hydroxybenzoate in this study is considered to be 1000 mg/kg body weight/day.

Shaw & deCantanzaro (2009): Subcutaneous injections of parabens occurred on each of days 1–4 of pregnancy from G0, commencing 3–6 h into the dark cycle. Injections occurred at four different sites to minimize any irritation resulting from paraben administration: right and left flank, rear middle area, and scruff of the neck. Female rats each received DMSO vehicle alone or either 35 or 40 mg propyl 4-hydroxybenzoate. The authors state this corresponds to a dose of ~1000 mg/kg/day though the weights of the animals are not reported. There was no apparent impact of propyl 4-hydroxybenzoate on the number of implantation sites at either dose. This was a non-GLP study.

Oishi et al. (2002): A 4-week repeat-dose study conducted on 21 days old juvenile male Wistar rats exposed at doses of 0.01, 0.1 or 1% propyl 4-hydroxybenzoate (99% purity) in the diet showed an effect on spermatogenesis. A decrease in the testicular and epididymal quantity of spermatozooids was observed with a lowest-observed adverse effect level (LOAEL) of 0.01% corresponding to an average propyl 4-hydroxybenzoate intake of 12.4±3 mg/kg/day. A dose-dependent decrease in serum testosterone concentration was significant at a dose of 1%, corresponding to 1290±283 mg/kg/day propyl 4-hydroxybenzoate. It has to be noted that this was a non-GLP, non-guideline study with small group size. There were a number of control values in parameters that were well outside of the normal range. The data were not consistent with literature data and data from other studies of Oishi for daily sperm production (DSP), epididymal sperm counts and

testosterone concentration, and there was no dose-response for the effect on DSP. In addition, a full study protocol and raw data are no longer available (Snodin, 2017).

Gazin et al. (2013): The objectives of the main reproductive toxicity study (Ricerca Biosciences, 2012d) were to determine the toxicity of the test item propyl 4-hydroxybenzoate following daily oral administration to juvenile male Wistar rats from the age of weaning on post-natal day (PND) 21 through sexual maturation and up to 11 weeks of age (8-week treatment period). The selected treatment period covers the juvenile (PND 21-35), peri-pubertal (PND 35-55), pubertal (55-70) and early adult stages in the male rat. As in the Oishi (2002) study, the study was performed in the same strain of juvenile male rat (Wistar Crj: WI (Han) and treatment started on PND 21. However, the duration of exposure was extended from 4 to 8 weeks (PND 77) and gavage (once daily) was used instead of dietary administration. Furthermore, a fourth dose level-group (low dose) was included in an attempt to determine a NOAEL. Additional animals were included to evaluate the reversibility of any toxic signs during a 26-week treatment-free period (to cover 3 spermatogenic cycles). Additional endpoints such as histopathology and serum LH and FSH levels were included in order to determine the mechanisms of the awaited testicular and epididymal effects. The pathology data and evaluation were subjected to an external review. The vehicle was 1 % (w/v) hydroxyethylcellulose 80-125 centipoises at 2 % in water for injection. Purity of the test substance, stability in the vehicle and homogeneity of the test suspension were controlled. The test item was applied once daily by gavage and Group 1 animals (controls) received the vehicle alone. For the analysis of testosterone, LH and FSH, blood samples of about 2 ml were taken from the retro-orbital sinus of all animals under isoflurane anaesthesia from the animals fasted for at least 14 hours in the morning of PND 78 and PND 79. Study results: No unscheduled deaths were observed. Clinical signs were restricted to transient post-dose hyper-salivation of animals of the high dose group, first noted on study day 9 (PND 30) and thereafter until the end of the treatment period, occasionally together with abnormal foraging. There was no influence of treatment on mean body weight gain in any group through to the end of the treatment period (study day 56) or treatment-free period (study day 237). Terminal mean body weight at the end of the treatment and treatment-free period was comparable with that in the concurrent control in all treated groups. There was no influence of treatment on time of sexual maturation of the males in any group. Mean body weights on the day of occurrence of balano preputial skinfold cleavage (in average on PND 43-44) were comparable in all groups. No influence of treatment on the levels of the measured hormones (LH, FSH and testosterone) was observed in any group. Isolated deviating findings were not dose-related and considered to be incidental. There were no effects of treatment on mean sperm counts and motility parameters at terminal sacrifice and sacrifice after the treatment-free period, apart from one single finding in the low-mid (10 mg/kg) dose group after the treatment period and one in the high dose recovery group. Both were associated with severe macroscopic and microscopic findings in testes or epididymes but were considered incidental because of the isolated occurrence. There were no body or organ weight differences that might indicate a treatment related effect. Occasional weight differences, including those with statistical significance between controls and treated animals were not dose-related and hence considered to be incidental or only to reflect normal individual variation. At the end of

the treatment period, the only effects of note were limited to minimal tubular atrophy/hypoplasia recorded in the right testis of three animals from the low dose group as well as in one animal from the high dose group. Severe tubular atrophy/hypoplasia of the right testis was sporadically recorded in one animal in the mid-low dose group, in correlation with soft testes in addition to small epididymides correlated with atrophy and aspermia. In summary of the pathology investigations, daily oral administration of propyl 4-hydroxybenzoate in post-weaning juvenile male Wistar rats for 8 weeks followed by a 26-week treatment-free period did not result in test item-related macroscopic or microscopic changes in the testes and epididymides. There was no evidence of any treatment-related effect on testicular and epididymal weights or on sperm count and motility data in any of the treated groups. In conclusion, the NOAEL of the study for male reproductive endpoints is 1000 mg/kg bw per day for the treatment period of 8 weeks. The present study did not confirm the effects on the reproductive functions reported by Oishi et al. (2002) and is regarded of sufficient quality to overturn the findings by Oishi, which is a study that suffers from numerous limitations as mentioned above.

4. CLP and Scientific Justification for No Classification

Clariant Produkte (Deutschland) GmbH notes that classification for reproductive toxicity according to CLP is not justified based on the scientific evidence in the context of the regulatory criteria (REGULATION (EC) No 1272/2008), and requests that the RAC reconsider the recommendation made by the dossier submitter.

According Regulation (EC) 1272/2008 of the European Parliament and of the Council („CLP Regulation“), classification as a reproductive toxicant is made on the basis of an assessment of the total weight of evidence which means that all available information that bears on the determination of reproductive toxicity is considered together in that both, positive and negative results, are assembled together into a weight of evidence determination

For an effect to warrant classification, CLP criteria primarily require, that the effect is adverse (CLP Annex I, section 3.7.2.1.1), which is furthermore characterized by several additional criteria including the assessment of the biological and toxicological significance, as well as the nature, severity, and incidence (CLP Annex I, section 3.7.2.3.1) of the effect. Furthermore, conclusions on the inherent ability of a chemical to induce a specific adverse effect (CLP Annex I, section 3) should be based upon the available data and an assessment of total weight of evidence (CLP Annex I, section 3.7.2.3.1) which includes assembling together both positive and negative results. As already described, the extensive scientific evidence from animal studies involving oral exposure to propyl 4-hydroxybenzoate demonstrates a lack of adverse reproductive effects per the CLP criteria (as detailed in section 3.7.2. of Annex I of the CLP) and therefore classification for development and fertility effects is not required.

To conclude on a classification determination, there is a need to take into account the whole toxicological evidence for propyl 4-hydroxybenzoate in a robust weight of evidence approach to develop an informed

regulatory decision that is commensurate and proportionate with all available data. Following these principles the following can be concluded with regard to the concerns brought forward by the CLH-dossier submitter:

Regarding AGD an isolated consideration without taking additional functional parameters into account is scientifically not appropriate or justified. AGD itself is not per se representing an adverse effect on development or reproduction. On the basis of above CLP criteria, the proposed classification proposal for propyl 4-hydroxybenzoate is therefore unjustified since all functional parameters (fertility and mating index) related with male reproduction were not affected in all available GLP and OECD conform high quality studies (OECD 443, OECD 422, OECD 414) up to the limit dose of 1000 mg/kg bw per day. The marginal changes in AGD were not accompanied by any expectable functional developmental or reproductive finding, were well within the range of historical control data, and no dose dependency was observed. Additionally, the results from the EOGRTS clearly demonstrate that *in utero* exposure to propyl 4-hydroxybenzoate up to the limit dose of 1000 mg/kg bw per day did not induce any functional, morphological or histopathological abnormalities in male reproductive organs.

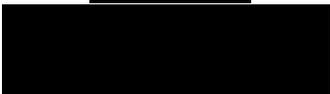
Regarding sperm parameters, based on the data of the EOGRTS, the slight increase in abnormal sperm morphology and the slight decrease in sperm motility were well within the range of historical data and without statistical significance and can therefore not be considered as test item related but as a reflection of biological variability. The adverse effect linked to a sperm is a functional impairment of male fertility. However, the results of the EOGRTS and OECD TG 442 study clearly showed that up to the limit dose of 1000 mg/kg bw per day that none of the parameters related to male reproductive performance and/or fertility were affected. A robust weight of evidence analysis which includes a dose-response analysis as requested by the CLP Regulation, i.e. the precision of the measurement in question, the range of the biological (natural) variation, the effect size, the statistical significance, the relationship to other effects related to the findings, and the weight of evidence coming from other studies, therefore fails to establish an adverse nature of the discussed AGD finding.

The CLH-dossier submitter raised a concern regarding male fertility after propyl 4-hydroxybenzoate exposure based on data provided by Oishi et al. (2002). However as already discussed, a scientific evaluation clearly indicates limitation of this non-GLP and non-OECD study (e.g. limited group size, limited evaluation of systemic toxicity, short period of treatment, absence of dose-response for daily sperm count). More importantly, the reported data on daily sperm production and epididymal sperm by Oishi and coworkers in itself is not consistent and/or not reproducible based on data from other studies (Gazin et al. 2013).

Summary of the Scientific Justification for no classification

Clariant Produkte (Deutschland) GmbH notes that an isolated consideration of effects on single endpoints which are lacking statistical significance, and which are without any dose dependency, is not appropriate and/or justified for classification as developmental and reproductive toxicity. More importantly, all findings of concern discussed by the evaluating MS Belgium are well within the range of the historical control data and thus represent biological variation rather than a substance related true toxicological significant effect. It is therefore concluded that the classification proposal for reproductive toxicity according to CLP is not justified based on an evaluation of the overall scientific evidence in the context of the regulatory criteria according Regulation (EC) No 1272/2008 of the European Parliament and of the Council),

Considering the CLP Regulation (Annex I: 3.7.1.3), adverse effects on sexual function and fertility include effects on the onset of puberty gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence or modifications in other functions that are dependent on the integrity of the reproductive systems. Based on data from the EOGRTS (Klimisch 1) no adverse effect on all above mentioned functional developmental and reproductive parameters up to the limit dose of 1000 mg/kg bw per day could be observed and thus a classification as Repr. Cat 2 is not justified.

Dr. 


Toxicologist GT/ ERT

Global Toxicology & Ecotoxicology - Global Product Stewardship

Clariant Produkte (Deutschland) GmbH

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Annex I - Summary of EOGRTS on propyl 4-hydroxybenzoate

The aim of this study was to assess possible adverse effects of the test item Propyl 4-hydroxybenzoate via oral administration (gavage) after repeated exposure during all phases of the reproductive cycle including pre and postnatal development with sexual maturation, the integrity of the male and female reproductive systems and systemic toxicity in males, pregnant and lactating females and young/adult offspring (development, growth, survival, and functional endpoints). In a single study, it tests parental (P) fertility and reproductive function, and offspring (F1) development through sexual maturity, including assessment of sexual landmarks (Cohort 1), nervous system - neuropathological and behavioural endpoints (Cohort 2), immune function (Cohort 3), learning and memory (Cohort 4) and effect on F2 generation by mating of F1 offspring if there are indications of potential adverse effects on F1 offspring. The P-generation groups contained thirty sexually matured animals per sex in control and HD group and 25 per sex in LD and MD groups. The test item formulation was prepared freshly once at least every 4 days and the prepared formulations were stored protected from light at 2-8 °C. The test item was suspended in 1 % aqueous hydroxyethyl-cellulose. Dose volumes were adjusted individually based on recent body weight measurements. The test item was administered daily in graduated doses to 3 groups of test animals at dose volume of 5 mL/kg bw. Animals of the control group were handled identically as the dose groups, but received 1 % aqueous hydroxyethyl-cellulose (viscosity 80-125 cP, 2 % in water at 20 °C), the vehicle used in this study.

The following doses were evaluated:

Control (C): 0 mg/kg body weight/day

Low Dose (LD): 100 mg/kg body weight/day

Medium Dose (MD): 300 mg/kg body weight/day

High Dose (HD): 1000 mg/kg body weight/day

The P-generation animals were exposed with the test item by oral gavage 2 weeks during pre-mating (males and females), 2 weeks during mating (males and females), 6 weeks post mating up to termination after weaning- 10 weeks total treatment (males), during pregnancy and lactation up to termination after weaning- 8-10 week's total treatment (females). At weaning, selected F1 offspring were assigned to specific cohorts for the investigations comprising sexual maturation, reproductive organ integrity and function, neurological and behavioural endpoints, and immune functions. In F1 males and females, the direct exposure to test item was started at weaning until the scheduled termination, i.e., until an age of 13 weeks (Cohort 1A, twenty animals per sex and group) or until study termination (weeks 20-25: Cohort 1B, twenty animals per sex and group).

Furthermore, Cohort 2A animals were sacrificed at an age of 12 weeks (Cohort 2A, ten animals per sex and group). Cohort 2B animals served for developmental neurotoxicity and were sacrificed at weaning (ten animals per sex and group). Cohort 3 animals underwent evaluation of developmental immunotoxicity and were sacrificed at an age of 8-10 weeks (ten animals per sex and group). Cohort 4 contained ten animals per groups and sex for learning and memory testing that were sacrificed after completion of the test on post-natal day 38-39.

During the period of administration, the animals were observed precisely each day for signs of toxicity. Animals that died were examined macroscopically and at the conclusion of the test, surviving animals were sacrificed and observed macroscopically. Once before the first exposure, and once a week thereafter, detailed clinical observations were made in all P animals and all cohorts (except cohort 2B and cohort 4). Body weight and food consumption were measured in all animals at specific intervals.

After 2 weeks pre-mating period, parental male and female from the same dose group were mated (1:1 pairing). F1 males and females from Cohort 1B were bred (1:1 pairing) after minimum treatment up to PND 90 to obtain a F2 generation.

Each F1 and F2 litter was examined as soon as possible after the delivery of the dam (PND 0) to establish the number and sex of pups, stillbirths, live births, runts and the presence of gross abnormalities. Live pups were counted, sexed and litters weighed within 24 hours of parturition (day 0 post-partum) and on days 4, 7, 14 and 21 postpartum. The anogenital distance (AGD) of each F1 and F2 (Cohort 1B) pup was

measured on PND 0 and all male pups were checked for the presence of nipples/areolae on PND 12. All selected F1 male and female pups from all cohorts (except cohort 2B and cohort 4) were checked daily for balano-preputial separation or vaginal patency, respectively starting from PND 30 in males and PND 25 in females. Vaginal smears of parental females were examined 2 weeks before beginning of treatment period, during 2 weeks pre-mating period and until the confirmation of mating. Vaginal smears were examined daily for all F1 females in cohort 1A after the onset of vaginal patency until the first cornified smear was recorded. Vaginal smear in cohort 1A was also examined for 2 weeks starting from PND 75. The vaginal smear in cohort 1B was examined during mating period to confirm the evidence of mating. Haematological, coagulation, thyroid hormone analysis (T4 and TSH) and clinical biochemistry parameters were determined with blood samples obtained from 10 randomly selected parental males and females and 10 randomly selected F1 male and female animals of cohort 1A at their terminal sacrifice. A urinalysis was also performed on samples collected from these animals prior to or as part of their terminal sacrifice. Thyroid hormone analysis (T4 and TSH) was also performed on 10 pups/sex/group at PND 4 and after weaning (pups not allocated to cohorts) on PND 22. To evaluate possible toxic effects on male fertility, sperm motility and testicular sperm head count was performed at the end of the treatment period from all parental generation males and all cohort 1A males of each group by using Hamilton Thorn sperm analyser. Sperm morphology was evaluated at the end of the treatment period from all parental generation males and all cohort 1A males from control and high dose groups. Ten male and 10 female cohort 2A and 2B animals from each treatment group were used for neurotoxicity assessments. Cohort 2A from each treatment group was subjected to auditory startle, functional observational battery, motor activity, and neuropathology assessments. An auditory startle test was performed on PND 24 (± 1 day) using animals in cohort 2A. The cohort 2A animals (between PND 63 and PND 75), were subjected to multiple detailed behavioural observations using functional observational battery of tests and test of motor activity. Cohort 4 included 10 males and 10 females from control and HD group for learning and memory testing between PND 21-42 by using Y water maze. Cohort 3 animals of 10 male and 10 female (on PND 56 ± 3 days), from each treatment group were used for a T-cell dependent antibody response assay to measure KLH-specific IgM and IgG antibodies. Pre- and postnatally induced immunotoxic effects at termination from 10 male and 10 female cohort 1A animals from each treatment group was evaluated by analysis of splenic lymphocyte subpopulation (CD4⁺ and CD8⁺ T lymphocytes, B lymphocytes, and natural killer cells) using one half of the spleen. For determination of test item plasma level, blood was taken from 10 animals/group/sex of all groups of parental generation (P), in cohort 1A and F2 pups of cohort 1B animals on PND 21 and or before final necropsy. In addition in F1 pups on PND 4 and PND 21- 22 blood was also sampled in 10 animals/group/sex at time of necropsy of the pups. On PND 13 (only control and HD) and 5 animals/sex, blood was sampled. Blood from F2 pups (10 pups/group/sex) from cohort 1B animals on PND 4 was also sampled for determination of test item plasma level. At the conclusion of the treatment period of parental animals and various cohorts, all animals were sacrificed and subjected to necropsy. The wet weight of a subset of tissues was taken and a set of organs/tissues was preserved. Animals that died or were sacrificed in a moribund condition were examined macroscopically and histopathologically. A full histopathological evaluation of the collected tissues was performed on high dose and control parental and cohort 1A animals. The histopathological examination of cohort 1A female ovaries included quantitative evaluation of primordial and small growing follicles and corpora lutea. From cohort 1A males, for the testes, a detailed qualitative examination was made taking into account the tubular stages of the spermatogenic cycle at evaluation of additional hematoxylin-PAS (Periodic Acid Schiff) stained slides. These examinations were not extended to animals of the other dosage groups as no treatment-related changes were observed in the HD group. Any gross lesion macroscopically identified was examined microscopically in all animals including found dead or moribund sacrificed animals. Neurohistopathology was performed for all control and high dose cohort 2A animals sacrificed after PND 90. Brain histopathology was performed for all control and high dose cohort 2B animals sacrificed on PND 21.

Summary Results

Mortality

In parental generation, from LD group, 3/25 females and from MD group 1/25 female was euthanised for animal welfare reasons. In cohort 1A, 2/20 HD males, 1/20 MD male and 2/20 HD females were found

dead. In cohort 1B, 1/20 control female was sacrificed in a moribund condition due to accidental femur fracture. There was also 1/20 control male and 1/20 MD male found dead during the study period. In cohort 2A, 1/10 MD female was sacrificed in moribund condition due to animal welfare reasons. In cohort 3, 1/10 LD male, 1/10 HD male, 1/10 LD female and 1/10 HD female were found dead during the study period. The decedents were randomly distributed and, hence, death was not deemed to be related to treatment with the test item. There were no gross lesions and histological findings that could be attributed to treatment.

Clinical Observations

In terminally sacrificed parental male and female animals, predominant clinical signs transiently observed in the majority of HD animals and few MD parental females were increased salivation and moving the bedding. In terminally sacrificed males and females from various F1 cohorts, similar clinical signs like in parental animals were observed in the HD group but in fewer animals compared to parental animals. The clinical signs salivation and moving the bedding in males and females were observed immediately after the dose administration and therefore were considered to be a sign of a local reaction to the test item rather than a systemic adverse effect and has no toxicological relevance. None of the parental or cohort 1B females showed signs of abortion or premature delivery.

Detailed Clinical Observations

During the weekly detailed clinical observation, no biologically or toxicologically relevant differences between the groups were observed in the parental generation and F1 cohorts (1A, 1B, 2A and 3) during the entire study period. Occasional statistical significant differences were observed in few parameters (i.e. animals sleeping or moving in cage, changes in skin, response to handling, salivation, faeces consistency) in parental generation and F1 cohorts. However, these were either before initiation of treatment, not dose dependent/consistent or not biologically relevant and therefore these findings were considered to be incidental and not related to the treatment with the test item.

Body Weight Development

In both male and female parental animals, there was no test item treatment related effect observed on group mean body weight and body weight gain in the dose groups when compared with the controls. Occasionally and marginally but statistically significantly lower or higher weight gain in cohort 1A animals of LD and HD groups was seen without consistency between the genders and is not considered toxicologically relevant. Slight but statistically significantly lower body weight in males and females of the HD group is not considered toxicologically relevant as this was also observed at the start of treatment. Occasionally and marginally but statistically significantly higher weight gain in LD females and MD group males and females and higher and lower weight gain in male and female HD animals in cohort 1B was seen without consistency between the genders and is not considered toxicologically relevant. A slight but statistically significantly higher body weight in HD females during late gestation and lactation is not considered toxicologically relevant.

Occasionally and marginally but statistically significantly lower weight gain in LD and HD males of cohort 2A animals of dose groups was seen without consistency between the genders and is not considered toxicologically relevant. Occasionally and marginally but statistically significantly higher body weight gain in LD males of cohort 3 is not considered toxicologically relevant. In positive control animals slightly but statistically significantly lower or higher body weight gain were observed occasionally. This is not considered to affect the intended KLH-specific immune reaction in this group. In cohort 4 males and females, there was no effect observed on mean body weight and weight gain between the control and HD group. Besides above mentioned not biologically relevant statistical significant differences, there were no considerable differences in body weight or body weight gain in this study. Overall, in all parental and F1 cohorts animals, body weight and body weight gain remained unaffected by the treatment with test item and values were in the normal range of variation throughout the treatment period when compared to the control group.

Food Consumption

No test item related or statistically significant effect of Propyl 4-hydroxybenzoate on food consumption was observed in males and females of parental generation or F1 cohorts during the whole study period except for statistically significantly lower food consumption observed during day 36-43 in the MD and HD males of cohort 1A and statistically significantly higher group mean food consumption during day 29-36 in LD group females of cohort 1A when compared to the controls. Due to the lack of dose dependency or consistency, this effect on food consumption in cohort 1A animals was considered as incidental and not adverse.

Oestrous Cyclicity

Test item had no biologically significant effect on the estrous cycle analysed during the 2 week pre-treatment and the 2 week pre-mating period in parental animals. There were no relevant differences in the length or sequence of cycle stages between the treatment groups and the control group. In cohort 1A females, no statistically or biologically significant effect was observed on the time between vaginal opening and first estrous cycle in treatment groups when compared with the controls. In these animals no biologically significant effect was observed on the estrous cycle length or sequence of cycle stages between the treatment groups and the control group analysed from PND 75 for 2 weeks.

Litter Data

In parental females, there were no test item treatment related or statistically significant effects observed in treatment groups on litter data parameters like total number of pups born, number of male pups, number of female pups, sex ratio, number of live pups, stillbirth, runt on PND 0 as well as number of live pups, male pups, number of female pups and sex ratio on PND 4, 7, 13 and PND 21 when compared with the controls. From 1B females, litter parameters like group mean total number of pups born, number of male pups, number of female pups, sex ratio, number of live pups, stillbirth, runt on PND 0 as well as number of live pups, male pups, number of female pups and sex ratio on PND 4, 7, 14 and PND 21 remained unaffected and without statistical significant difference when compared with the controls.

Litter Weight Data

There was no test item related effect on pup mean weight, total litter weight, male and female litter weight on PND 0, PND 4, 7, 14 and PND 21 observed in parental and cohort 1B treatment groups when compared to the controls. There was no statistically significant change in dose groups compared to corresponding controls except statistically significantly lower pup mean weights from parental females on PND 14 in HD group. As total litter weight and mean pup weight in cohort 1A offspring, however, were not different from controls, this is not assumed to be toxicologically relevant.

Precoital Interval and Duration of Gestation

There was no test item related or statistically significant effect observed on the duration of precoital interval and the duration of gestation in the parental and cohort 1B female dose groups when compared to the respective control group.

Pre- and Post- Natal Data

There were no test item treatment related effects observed on the number of corpora lutea, implantation sites, live pups on PND 0, percent preimplantation loss and post implantation loss in parental and cohort 1B treatment group females when compared with the corresponding control group.

Reproductive Indices

There were no test item related effects observed on the reproductive indices (percent male mating index, female mating index, male fertility index, female fertility index, gestation index and live birth index) in parental and cohort 1B dose group animals when compared to the respective control group. The survival index of pups during PND 0 to 4, PND 4 after interim sacrifice to PND 13 and PND 13 after interim sacrifice to PND 21 in parental females and during PND 0 to 4, PND 4 after interim sacrifice to PND 14 and PND 14 to PND 21 in cohort 1B females remained unaffected and within the range of biological variation in treatment groups when compared with the respective controls.

Pup Survival Data

No test item related effect on mean mortality of pups from PND 0 to 4, PND 4 after interim sacrifice to PND 13 and PND 13 after interim sacrifice to PND 21 in parental females and from PND 0 to 4, PND 4 after interim sacrifice to PND 14 and PND 14 to PND 21 in cohort 1B female treatment groups when compared to the control group.

Anogenital Distance and Nipple Retention

In male pups from the parental generation, on PND 0 marginal shorter absolute but not relative anogenital distance (AGD) was observed in the HD group when compared to the controls. In the female pups from parental females on PND 0, minimal lower absolute and relative anogenital distance in LD, MD and HD groups was observed when compared with the controls. As all these differences were only very slight and values were within the range of historical control data this is not considered to be toxicologically relevant. In male pups from cohort 1B females on PND 0, statistically significantly lower absolute and relative AGD were observed in the HD group when compared to the controls. However, individual values were within the range of historical control data. In female pups from cohort 1B females on PND 0, no statistically significant effect was observed on any AGD or pup weight parameter. No effect of toxicological relevance was observed on nipple retention in the pups of any of the groups from parental and cohort 1B females when compared with the respective controls. Number of nipple retention in HD males from parental females was statistically significantly lower in males and statistically significantly higher in HD males from cohort 1B females when compared with the controls. Due to the inconsistent nature of the responses no toxicological significance is attributable to this observation. In addition, these findings were not associated with developmental or reproduction toxicological effects in cohort 1B animals.

Pup External Finding

No test item related gross external abnormalities of toxicological relevance on PND 0-20 were observed in the pups of any of the groups from parental and Cohort 1B females.

Haematology and Blood Coagulation

In 10 selected parental males, no test item related adverse effects were observed for haematological parameters in treatment groups when compared to the control group. Marginal but statistically significant differences, i.e. in LUC (large unstained cells) are without toxicological relevance. In 10 selected parental females, no test item related adverse effects were observed for haematological parameters in treatment groups when compared to the control group.

Marginal but statistically significant differences in HCT, WBC, MCHC and Neut are without toxicological relevance, due to their slightness or because of a lack of dosedependency. No test item related effect was observed on coagulation parameters in parental females when compared to the controls. Minimal but statistically significantly higher prothrombin time (PT) in the MD group when compared with the control which was not considered to be of toxicological relevance.

In 10 selected cohort 1A males per group sacrificed at the end of the treatment period, marginally but statistically significantly different HGB, WBC, basophils, platelets and neutrophils were observed in dose groups when compared with the controls. As the differences were slight, values within the range of historical control data or without dose dependency this is not assumed to be toxicologically relevant. In 10 selected cohort 1A females per group sacrificed at the end of the treatment period, marginally but statistically significantly different HCT, HGB, RBC, WBC, monocytes and basophils were observed in dose groups when compared with the controls. As the differences were slight, values within the range of historical control data or without dosedependency this is not assumed to be toxicologically relevant. No test item related effect was observed on coagulation parameters in cohort 1A males and females when compared with the respective controls. Marginal but statistically significantly higher group mean prothrombin time (PT) in HD females was observed when compared with the controls, but is not considered toxicologically relevant.

Clinical Biochemistry

There were few marginal but statistically significant differences in clinical biochemistry parameters of male and female animals of parental and cohort 1A. In parental animals differences in AP, TP, Urea, TBA, ALAT, Crea were observed and in cohort 1A animals potassium level was slightly altered. As they were within the range of historical control data, not dose-dependent, not consistent between the genders and did not coincide with histopathological findings, this is not considered toxicologically relevant.

Urinalysis

The urinalysis performed in 10 selected male and female animals per group from parental and cohort 1A sacrificed at the end of treatment period revealed no test item treatment related effect in treatment groups when compared with the controls and all urinary parameters were in the normal range of variation.

Thyroid Hormone (T4 and TSH) Analysis

Parental:

In parental males and females, group mean T4 and TSH levels were comparable with the controls except slight but statistically significantly higher group mean TSH values in HD group parental females. TSH level of few animals of this group were more prominently increased. However, as the variability of the individual data was high and the findings were not associated with any microscopic finding of hypertrophy or an increased weight of the pituitary gland. Thus, this is not assumed to be toxicologically relevant.

F1 pups on PND 4 and PND 21:

In pups sacrificed on PND, T4 and TSH levels in treatment groups were comparable with the controls. In pups sacrificed on PND 21, T4 levels in treatment groups were comparable with the controls.

Cohort 1A:

In males and females of cohort 1A, group mean T4 values were comparable with the controls. There was also a slight but statistically non-significant increase in group mean TSH values in HD group males when compared with the controls. As this was also not associated with any microscopic finding of hypertrophy or an increased weight of the pituitary gland, this is not assumed to be toxicologically relevant.

Sexual Maturity

No abnormalities of genital organs like persistent vaginal thread, hypospadias or cleft penis were observed in any of the pup.

Cohort 1A:

No significant difference in day of onset of vaginal opening in females and balanopreputial separation in males was observed in the presence of slightly lower body weight of the pups in MD and HD groups.

Cohort 1B:

No significant difference in day of onset of the vaginal opening in females, balanopreputial separation in males and body weights was observed in treatment groups when compared with respective controls.

Cohort 2A:

No statistically significant difference in group mean body weight and day of onset of the vaginal opening in females was observed in treatment groups when compared with controls.

Cohort 3:

No statistically significant difference in group mean body weight and day of onset of the vaginal opening in females and balano-preputial separation in males was observed in treatment groups when compared with respective controls.

Sperm Analysis

Motility:

Test item had no effect on epididymal sperm motility count analysed from parental and cohort 1A males at terminal sacrifice.

Sperm Morphology:

Parental and Cohort 1A males:

Evaluation of sperm morphology from control and HD parental and cohort 1A males did not reveal any indication for toxicity induced by the test item, and percentage of normal and abnormal sperms in treatment groups were comparable with the controls.

Testicular Sperm Head Count:

Parental and Cohort 1A males:

Test item had no effect on testicular sperm head count analysed from parental and cohort 1A males at terminal sacrifice.

Learning and Memory

Analysis of learning and memory data from cohort 4 males and females revealed that there were no test item related effect in escape latency time observed during the learning phase (PND 28/29) and memory phase on PND35/36 when compared to control in both genders by considering 6 rounds of mean escape latency.

Auditory Startle Response

Analysis of data from auditory startle test performed on PND 24 using male and female animals in cohort 2A revealed no toxicologically significant changes observed in mean startle response in animals treated with test item groups as compared to the control group.

Functional Observations

In males and females from cohorts 2A, no relevant or statistically significant effects were observed in any of the parameters of the functional observation battery during evaluation between PND 63 and 75 when compared with the controls. There were no biologically relevant differences observed in body temperature between the groups.

Motor Activity

Motor activity data analysis from cohort 2A males and females between PND 63 and 75 revealed no relevant effects on motor activity (animal movements and number of rearings) in treatment groups when compared with the controls.

Splenic Lymphocyte Subpopulation

There was a tendency towards higher lymphocytes, identified as T cells, especially CD4 and CD8 T-cell subpopulations in males and females of all dose groups of cohort 1A, when compared to controls. There was no indication of an immunosuppressive effect of the test item on lymphocytes sub-populations.

Antibody Assay (Immunogenicity)

Cohort 3

Results reported here were obtained using a KLH concentration of approx. 0.06 mg/kg. Thus, the dose that was applied to the animals intravenously was approx. only 20 % of the intended and validated dose of 0.3 mg/kg bw. Therefore, although visible in the data, the immunological stimulus used in this study was weaker than intended which has to be taken into consideration when interpreting the test results. An expected immunosuppressive effect after immunization with KLH was shown after administration of positive control substance cyclophosphamide in female animals (based on IgM and IgG values). In male animals of this group KLH-specific IgM levels were lower than in negative controls, however, they were mostly slightly increased when compared to pre-immunization levels. Nevertheless, even under consideration of lower KLH-specific IgG antibody levels an immunosuppressive effect could also be demonstrated in males of this positive control group.

In almost all male animals of the test item dose groups KLH-specific IgM (day 6) and IgG (day 14) level responses were higher than before immunization and also higher than in the positive control group but lower than in the negative control group. Although the interpretation of the data with regard to a potential immunosuppressive effect is hampered this demonstrates a functioning immune system. Due to the administered lower KLH concentration and the variability of data, interpretation of the data is only possible to a limited degree but does not point to a biologically meaningful immunosuppressive effect of the test item in male animals. In females of the test item dose groups, specific IgM levels following KLH administration compared to before immunization were increased in less animals and not quite as clear as in the negative control group. However, KLH-specific IgG levels in these animals were more similar to negative control animals which

indicates a functioning immune system. Increased total IgM and IgG serum levels in these animals indicate a normal immune response to administration with the immunogen.

Overall, the results obtained allow to conclude the correct functioning of the immune system. This conclusion is supported by the absence of any other effect on the immune system in this study (clinical observations including body temperature measurements, phenotyping of splenocyte subpopulations, clinical pathology parameters, macroscopic and histopathological evaluation of lymph nodes, peyer patches, spleen and thymus) which provides strong evidence with regard to the absence of an immunotoxic effect. The observed findings did not show any clinical relevance or signs of an impaired functioning of the immune system and are thus not considered to be toxicologically relevant.

Determination of Test Item Plasma Level

The systemic exposure to propyl 4-hydroxybenzoate (test item) and 4-hydroxybenzoic acid (metabolite) assessed from parental and cohort 1A male and female rats (30 ± 10 minutes post dose) were demonstrated at LD (100 mg/kg/day), MD (350 mg/kg/day) and HD (1000 mg/kg/day) groups. Whereas in parental animals the test item was detected at low concentrations or below the level of quantification, the metabolite was found in almost all dosed animals at high concentrations. In addition, exposure to propyl 4-hydroxybenzoate (test item) and 4-hydroxybenzoic acid (metabolite) was demonstrated in F1 pups (PND4, 13, 21), Cohort 1B-F2 pups (PND4, 21) and Cohort 4 pups (PND38/39). Exposure at PND4 demonstrated transfer of test item via milk.

Pathology

Parental and all cohorts:

Few spontaneous gross pathological changes were recorded for male and female animals from parental generation and various cohorts and were not considered to be treatment-related.

Organ Weight

Slight changes in organ weights observed in parental generation and in cohorts 1A, 1B, 2A, 2B, i.e. in liver, prostate gland with seminal vesicles, thymus, heart and adrenal glands were not consistent and not associated with histopathological findings. In parental males, statistically significantly lower absolute and relative prostate with seminal vesicles and relative liver weights in HD group were observed when compared with the controls. In parental females, there were no statistically significant and toxicologically relevant difference in the absolute and relative organ weights of the dose groups when compared to the control group. In cohort 1A males, statistically significantly lower absolute and relative liver weights in MD, absolute liver, heart and adrenal gland weights in HD group were observed when compared with the controls.

In cohort 1A females, there were no statistically significant differences in the absolute and relative organ weights of the dose groups when compared to the control group. Weights of lymph nodes, spleen and thymus of cohort 1A animals revealed no considerable changes that could indicate a test item related immunotoxic effect. Organ weight from male and female cohort 1B animals remained unaffected due to treatment with the test item. There was no effect on brain weights in male and females of cohort 2A and 2B used for assessment of neurotoxicity. There was no effect on brain, spleen and thymus weights in F1 pups not selected for cohorts and F2 pups from cohort 1B females at weaning.

Histopathology

P-generation animals were sacrificed during the course of the study. The decedents were randomly distributed and, hence, death was not deemed to be related to treatment with the test item. There were no gross lesions and histological findings that could be attributed to treatment. Non-pregnant females were randomly distributed throughout the groups including controls. No specific findings were noted that could be related to infertility, and again these cases were not related to treatment with propyl 4-hydroxybenzoate.

In the F1-generation, there were also few animals died during the course of the study. In a few cases, the cause of death may have been related to gavage accidents. No gross lesions nor histological lesions could be attributed to treatment with the propyl 4-hydroxybenzoate. Neuropathology evaluation and evaluation of reproductive organs did not reveal any induced lesion.

Dose Formulation Analysis

Formulation analysis for concentration verification and homogeneity was performed on collected samples at various intervals during the study. Nominal concentrations were confirmed for all dose groups, as measured concentration did not differ from nominal concentration by more than 15 %. All samples were homogenous, as COV was below or equal to 10 %.

Conclusion

On the basis of the present study, the Extended One-Generation Reproductive Toxicity Study after oral administration in male and female Wistar Rats with propyl 4-hydroxybenzoate with dose levels of 100, 300, and 1000 mg/kg body weight day the following conclusions can be made:

General toxicity:

Few mortalities/morbidities were randomly distributed throughout the majority of groups of parental animals and various cohorts. Based on histopathology the cause of death was not evident in most animals whereas in few, the cause of death could be related to the technical gavaging error and not due to systemic toxicity caused by the test item. There were no clinical signs of toxicological relevance observed in the treatment groups.

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**Statement to CLP Report
Concerning
Extended One-Generation Reproductive Toxicology
Study (OECD 443)
with
Propyl 4-Hydroxybenzoate
(BSL Study No. 176898)**

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Table of Contents

	page
1. Objective	3
2. Points of Discussion	4
2.1. Sperm Motility in F0 Parental and F1 Cohort 1A and Sperm Morphology in F0 Parental (Tail Only) and F1 Cohort 1A (Abnormal Sperm)	4
2.2. Precoital Interval in F1 Cohort 1B	5
2.3. Post-Implantation Loss in F0 Parental	5
2.4. Testis Weight in F1 Cohort 1A	6
2.5. AGD in F1	6
2.6. Nipple Retention in F1	7
3. Concluding Statement	8
4. References	9

1. Objective

In this document data of specific end points of the Extended One-Generation Reproductive Toxicology Study (OECD 443) on Propyl 4-Hydroxybenzoate (BSL study no. 176898) referred to in the CLP report "Proposal for Harmonized Classification and Labelling" for propyl 4-hydroxybenzoate, based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2 issued by FPS Public Health, Food Chain Safety and Environment, Brussels, are discussed and a statement is issued.

The statement addresses a disconsensus on interpretation of results in fertility/developmental toxicity end points ultimately leading to different NOAELs, where in the CLP report a NOAEL for male fertility at 300 mg/kg bw/d instead of 1000 mg/kg bw/d (as in study report 176898) is proposed whereas in the study report a NOAEL for developmental and reproductive toxicity, neurotoxicity and immunotoxicity of 1000 mg/kg bw/d is given.

The respective end points are:

- Sperm motility in F0 parental and F1 cohort 1A
- Sperm morphology in F0 parental (tail only) and F1 cohort 1A (abnormal sperm)
- Precoital Interval in F0 parental and F1 cohort 1B
- Post-Implantation Loss in F0 parental
- Testes Weight in F1 cohort 1A
- AGD in F1
- Nipple Retention in F2

2. Points of Discussion

2.1. Sperm Motility in F0 Parental and F1 Cohort 1A and Sperm Morphology in F0 Parental (Tail Only) and F1 Cohort 1A (Abnormal Sperm)

In respect to Sperm Motility CLP report states: "Male reproduction parameters were examined and revealed a reduction of sperm motility at the highest dose."

According to study report "Test item had no effect on epididymal sperm motility count analysed from all males at terminal sacrifice by using Hamilton Thorn Sperm Analyser (TOX IVOS Version 13.0C). Although group mean motility values from parental and cohort 1A males were marginally lower in HD group, this effect was attributed to very low values from one each parental and cohort 1A male and sperm motility values from all other males were comparable with control and therefore this marginal decrease in group mean motility in the HD group was not considered as adverse."

It should be added that sperm motility was not statistically significantly different between dose groups and control group, neither in F0 parental nor in F1 cohort 1A.

Moreover, sperm motility results were within the range of the lab's Historical Control Data (HCD) (mean: 83.83% with a \pm SD range between 72.83 and 94.84%). Although the mean of the HD group (Mean 72.67% with SD 19.50%) was marginally lower than the HCD range, it should be noted that all other groups, including Controls were also on the lower side of the HCD range. (Means between 77.05% and 77.98%).

When looking at individual F0 parental values, a single HD group animal can be identified with a very low motility value (animal no. 92; 0% motility, correlated with histopathological finding of aspermia). However, this is also the case for control animal no. 22 (10.5% motility, correlated with histopathological finding of tubular edema), strongly suggesting an inherent strain-specific background incidence of testicular findings in animals with very low or absent sperm motility. These lead to large standard deviations and subtle differences between groups have to be interpreted carefully.

In respect to Sperm Morphology of F0 generation CLP report states: "Sperm morphology was critically affected regarding the only tail sperm which reached a percentage of 8.17% at the highest dose compared to 2.96 % only in the control group, corresponding to an increase of approx. 276 %."

The study report states: "Evaluation of sperm morphology from control and HD parental and cohort 1A males did not reveal any indication for toxicity induced by the test item, and percentage of normal and abnormal sperms in treatment groups were comparable with the controls."

Parental HD group animal no. 92 was also affected in respect to sperm morphology, showing increased numbers of "Tail only" finding, as mentioned in the CLP report. This finding occurred 116 times in this animal whereas the remaining 17 animals of this group showed a total of 85 counts. Thus, like in the case of sperm motility, the higher mean of "Tail only" finding relies on a single animal that – as stated above – can potentially be regarded as background incidence. Control animal no. 22 was not evaluated for sperm morphology.

Regardless of sperm motility and morphology findings, animal no. 92 was mated with female no. 202 which was sperm positive on the 5th mating day and gave birth to a litter of 11 alive pups.

The higher percentage of abnormal sperm in the F1 cohort 1A cohort (HD group 9.53% vs. C group 5.18%) is slightly above the HCD range (Mean 4.90% with a 2SD range between 0.52% and 9.27%). This is related to animal no. 298 showing 67% abnormal sperms. This animal also showed markedly low percentage of motile sperm, markedly high percentage of static sperm, markedly low percentage of rapid sperm, a low testis weight (see below) and histopathologically aspermia in the epididymides and tubular degeneration in the testes. When not considering HD animal no. 298, the rate of abnormal sperms in the HD did not considerably differ from Control group (HD 5.81% vs. C 5.18%) suggesting that this is possibly an incidental finding. Generally, it should be noted that differences in sperm motility were smaller in F1 compared to F0 parental generation.

Finally, it should be noted that neither in the F0 parental nor in F1 cohort 1A were there any indications showing that a tendency towards lower sperm motility or increased morphological findings

are associated with an effect on fertility and development of the respective next generation. This refers to reproductive indices as percent male mating index, female mating index, male fertility index, female fertility index, gestation index and live birth index and duration of gestation, survival index of pups, litter data and pre- and post natal data (pre- and post implantation loss, anogenital distance, nipple retention as well as precoital interval see below).

Therefore, the tendency towards lower sperm motility and the sperm morphological finding of "tail only" is not assumed to be a clear toxic sign with respect to male fertility.

2.2. Precoital Interval in F1 Cohort 1B

CLP report states in respect to F0 parental: "However, precoital interval was slightly higher in all tested doses in comparison with the controls." CLP report states also in respect to F1 cohort 1B: "Regarding female reproduction parameters, precoital interval examination exhibited a dose-response increase as the parameter was of 1.94, 2.20, 2.74 and 2.83 days, resp. at 0, 100, 300, 1000 mg/kg bw/d."

The study report states: "There was no test item related or statistically significant effect observed on the duration of precoital interval and the duration of gestation in the parental and cohort 1B female dose groups when compared to the respective control group."

It should be added that means were in the range of HCD in F1 cohort 1B dose groups (HCD mean: 2.31 days with a SD range between 0.83 days and 3.79 days). Admittedly, a slight tendency in precoital length can be seen. However, no difference was seen in estrous length in F1 cohort 1A, mating/sexual behaviour, gestation length or in any other pre- or postnatal parameters in F1. Therefore, this is seen as an isolated finding, possibly related to olfactory cues from the test item formulations. There is no indication for considering it as an adverse effect in respect to reproductive toxicity, in this study.

2.3. Post-Implantation Loss in F0 Parental

CLP report states in respect to F0 parental: "Furthermore, percentage of post-implantation loss was increased at the highest dose (approx. 149% compared to the control group)."

The study report states: "There were no test item treatment related effects observed on the number of corpora lutea, implantation sites, live pups on PND 0, percent pre implantation loss and post implantation loss in parental and cohort 1B treatment group females when compared with the corresponding control group."

It should be added that the mean of the HD group (8.98%) was in the range of HCD (mean: 10.18% with a \pm SD range between 0 and 26.75%).

Moreover, looking at the individual data in the HD group it becomes evident, that a single dam showing 100% post-implantation loss is responsible for the higher mean. Post-implantation loss of the remaining animals was in the range of control animals. The fact that complete implantation loss was also observed in a control animal of F1 cohort 1B (no. 479) suggests that the finding of complete implantation loss in F0 parental HD group is incidental. Neither the number of implantation sites nor the number of alive pups was lower in the F0 parental HD group when compared to the controls. This is reflected in the absence in a considerable difference of prenatal loss (according to [4] (no. alive pups / no. implantation sites: HD 93.33% vs. C 93.75%).

Finally, no effect of Propyl 4-Hydroxybenzoate on post-implantation loss was observed in the F1 Cohort 1B animals of the study, as percentage was even lower in the HD group (7.61%) when compared to C group (9.05%).

Therefore, the tendency towards higher percentage of post-implantation loss in F0 parental HD group is not assumed to be toxicologically relevant.

2.4. Testis Weight in F1 Cohort 1A

CLP report states in respect to F1 cohort 1A: "At the highest dose, absolute testes weight was decreased (1.817, 1.782, 1.839 and 1.677 g, resp. at 0, 100, 300 and 1000 mg/kg bw/d)."

In the study report no considerable testes weight changes are mentioned. For clarification: the weight mentioned in the CLP report is from the left testis used for sperm analysis. The organ weight tables of cohort 1A include data on the right testis. The latter table also includes a calculation of the testis weight normalized to body weight. Neither in right (absolute and normalized) nor in left (absolute) testis weight was there a statistically significant difference between dose groups and control group. Comparison between absolute mean weight (HD 4.9329 % below controls) and normalized weight (HD 1.8223 % above controls) shows that the decrease coincides with a lower body weight in this group, when compared to controls (at necropsy on day 64 mean body weight of F1 cohort 1A HD animals was also approx. 8 % below controls). As, body weight generally correlates well with testes weight [1], we assume that this is no specific sign of reproductive toxicity but a consequence of a slightly lower body weight gain.

Finally, no considerable difference in absolute or normalized testes weight was observed in F1 cohort 1B dose groups (HD group 3.9871% and 4.7635 % above controls, respectively) and no considerable difference in absolute or normalized testes weight was observed in F0 parental dose groups (HD group 1.1162% and 2.7495 % above controls, respectively).

2.5. AGD in F1

The CLP report states in respect to F1 "However, anogenital distance and nipple retention were significantly changed. In males, modification was noted at the highest dose, whereas, in females, change was observed in all treated groups and was dose-related."

The study report states: In male pups from the parental generation, on PND 0 marginal shorter absolute but not relative anogenital distance (AGD) was observed in the HD group when compared to the controls. In the female pups from parental females on PND 0, minimal lower absolute and relative anogenital distance in LD, MD and HD groups was observed when compared with the controls. As all these differences were only very slight and values were within the range of HCD this is not considered to be toxicologically relevant. In male pups from cohort 1B on PND 0, statistically significantly lower absolute and relative AGD were observed in the HD group when compared to the controls. However, individual values were within the range of HCD. In female pups from cohort 1B females on PND 0, no statistically significant effect was observed on any AGD or pup weight parameter.

It should be added that the HCD for relative anigenital distance (AGD) of males is: 1.44 with a \pm SD range between 1.24 and 1.65). Relative AGD in male F1 pups was 1.51, 1.48, 1.46 and 1.46, resp. at 0, 100, 300 and 1000 mg/kg bw/d. Relative AGD in male F2 pups was 1.61, 1.55, 1.55 and 1.52, resp. at 0, 100, 300 and 1000 mg/kg bw/d. Thus, all means were well within range of HCD. All data refer to PND 0.

The HCD for relative anigenital distance (AGD) of females is 0.58 with a \pm SD range between 0.29 and 0.86). Relative AGD in female F1 pups was 0.68, 0.62, 0.61 and 0.61, resp. at 0, 100, 300 and 1000 mg/kg bw/d. Relative AGD in female F2 pups was 0.58, 0.55, 0.54 and 0.59, resp. at 0, 100, 300 and 1000 mg/kg bw/d. Thus, all means were well within range of HCD. All data refer to PND 0.

In male pups of F0 parental and F1 generations the decreases in mean rel. AGD were 3.3% (F1) and 4.6 % (F2) below controls. Decreased AGD is indicative of anti-androgenic effect in male animals and often correlated with hypospadias, cryptorchidism or undescendent testes [2],[3],[5]. These phenotypic signs were not observed in this study. Furthermore, in case of male F1 animals there was also no indication of an effect on developmental endpoints, e.g. day of balano-preputial separation or on fertility of cohort 1B. Similarly, in females of F1 generation no indication of disturbed vaginal patency, estrous cyclicity or fertility of cohort 1B was seen. Therefore, the slight effect on AGD was not considered to be an adverse effect.

2.6. Nipple Retention in F1

In respect to F1 pups the CLP report states "However, anogenital distance and nipple retention were significantly changed. In males, modification was noted at the highest dose." In respect to F2 pups the CLP report states "However, as observed in F1 pups (produced from parental animals), anogenital distance and pup nipple retention was significantly affected."

The study report states: "No effect of toxicological relevance was observed on nipple retention in the pups of any of the groups from parental and cohort 1B females when compared with the respective controls. Group mean number of nipple retention in HD males from parental females was statistically significantly lower than in controls. In males from cohort 1B females, nipple retention was statistically significantly higher when compared with the controls. This higher incidence from 1B females was attributed to all pups with a higher incidence of nipple retention from just one dam (no. 540) and considered to be incidental and not related to the treatment with test item".

It should be added that the HCD for nipple retention of males is: 0.31 with a \pm SD range between 0 and 1.11). Nipple retention in male F2 pups was 0.33, 0.20, 0.42 and 0.68, resp. at 0, 100, 300 and 1000 mg/kg bw/d. Thus, all means were well within range of HCD. All data refer to PND 12/13.

A higher number of retained nipples in male pups is biomarker for anti-androgenic effect and in rodents is associated with adverse effects such as hypospadias and cryptorchidism, decreased penile length and reduced terminal vesicle weight in rats [6]. In respect to F1 generation no such findings were present in this study. As the higher mean of F2 nipple retention is mainly related to a single litter (after exclusion of this litter with 0.55 vs. 0.33 only a slightly higher mean remains in HD when compared to controls), this is not assumed to be adverse to development of male pups at the doses tested.

3. Concluding Statement

On the basis of above mentioned interpretation of the study results, I conclude – in agreement with the study director – a NOAEL for developmental and reproductive toxicity, neurotoxicity and immunotoxicity of 1000 mg/kg bw/d.

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