Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification:

Reaction mass of 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol and benzyl(diethylamino)diphenylphosphonium 4-[1,1,1,3,3,3-hexafluoro-2-(4-hydroxyphenyl)propan-2-yl]phenolate (1:1)

EC Number: -

CAS Number: -

Index Number: -

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Note on confidential information

Please be aware that this report is intended to be made publicly available. Therefore it should not contain any confidential information. Such information should be provided in a separate confidential Annex to this report, clearly marked as such.

CONTENTS

1 PHYSICAL HAZARDS

Not evaluated in this CLH proposal.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

See CLH dossier, no studies are included in Annex I.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

Not evaluated in this CLH proposal.

3.2 Acute toxicity - dermal route

Not evaluated in this CLH proposal.

3.3 Acute toxicity - inhalation route

Not evaluated in this CLH proposal.

3.4 Skin corrosion/irritation

Not evaluated in this CLH proposal.

3.5 Serious eye damage/eye irritation

Not evaluated in this CLH proposal.

3.6 Respiratory sensitisation

Not evaluated in this CLH proposal.

3.7 Skin sensitisation

Not evaluated in this CLH proposal.

3.8 Germ cell mutagenicity

Not evaluated in this CLH proposal.

3.9 Carcinogenicity

Not evaluated in this CLH proposal.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Study 1

Study reference: Study report 2011, 4,4'-(1,1,1,3,3,3-hexafluoropropane-2,2-diyl)diphenol: oral (gavage) combined repeated dose toxicity study with reproduction/developmental toxicity screening test in the rat (OECD 422 1996 with recovery groups) (2011).

Detailed study summary and results:

Test type

Guideline study, OECD Test Guideline 422, no deviations, GLP compliant.

Test substance

- Test material used in the study is Bisphenol AF (BPAF, EC nr: 216-036-7, CAS no: 1478-61-1).
- Degree of purity: Purity 99.69%.
- No impurities that affect the classification
- Batch number: 090607

Test animals

- Sprague-Dawley rats, males and females
- 12 males and 12 females per treatment group. Recovery animals: 5 males and 5 females per treatment
- Age at study initiation: ca. 9 weeks old
- Weight at study initiation: 301 - 375 g

Administration/exposure

- Route of administration – oral (gavage)

- Duration of exposure: Test groups and controls: Once daily for 55 consecutive days (including a 2 week maturation phase, pairing, gestation and early lactation for females). Recovery groups: treated for 42 consecutive days and then maintained without treatment for 14 days.
- Doses/concentration levels: 0, 30, 100, 300 mg/kg bw/day. Dose selection rationale: Dose concentrations were based on the findings of a preliminary study conducted at 1000, 400 and 100 mg/kg bw
- Vehicle: arachis oil. Concentration in vehicle: 7.5, 25 and 75 mg/mL prepared for 30, 100 and 300 mg/kg/day test groups. Amount of vehicle (if gavage): 4 mL/kg bw
- Preparation of dosing solutions:

  Test material was prepared at the appropriate concentration as a suspension in Arachis oil BP. Stability and homogeneity of formulations was verified in a previous study. Fresh formulations were prepared every 2 weeks and stored at ca. +4 ºC in the dark. Subsamples were taken from each formualtion to verify concentration using a validated HPLC method. Measured concentrations were within ± 9 % of the nominal concentration throughout the study.

Description of test design:

- Details on mating procedure*:* Non-recovery animals were paired on a 1 male: 1 female basis within each dose group, for a period of up to fourteen days. Cage tray-liners were checked each morning for the presence of ejected copulation plugs and each female was examined for the presence of a copulation plug in the vagina. A vaginal smear was prepared for each female and the stage of the oestrous cycle or the presence of sperm was recorded. The presence of sperm within the vaginal smear and/or vaginal plug in situ was taken as positive evidence of mating (Day 0 of gestation) and the males were subsequently returned to their original holding cages (unless required for additional pairing). Mated females were housed individually during the period of gestation and lactation.
- Premating exposure period for males and females (P) was 14 days.
- Dosing schedules and pre and post dosing observation periods for P: Time schedule: immediately before dosing, up to 30 mins after dosing, one and 5 hours after dosing, during the working week. Animals were observed immediately before dosing, soon after dosing and 1 hour after dosing, at weekends. During the treatment-free period, recovery animals were observed twice daily (once at weekends).
- Parameters assessed for P: Cage sides observations, detailed clinical observations, body weight, food consumtion and compound intake, food efficiency, water consumption and compound intake, hematology, clinical chemistry, urinalysis (males only), neurobehavioural examination, post-mortem examination.

- Parameters assessed for F1: Number of offspring born, Number of offspring alive recorded daily and reported on Days 0 and 4 post partum, Sex of offspring on Days 0, 1 and 4 post partum, Clinical condition of offspring from birth to Day 5 post partum, Individual offspring weights on Days 0 and 4 post partum. Post-mortem examinations.
- Oestrous cyclicity (P):Group mean values for oestrous cycles for test and control group animals were determined. Smears were taken and evaluated daily.
- Sperm parameters (P):Parameters examined in male parental (P0) generation: testis weight, epididymis weight
- Reproductive indices: The following parameters were calculated from the individual data during the mating period of the parental generation; Pre-coital interval - calculated as the time elapsing between initial pairing and the observation of positive evidence of mating, Fertility indices – mating index and fertility index calculated, Gestation length - calculated as the number of days of gestation including the day for observation of mating and the start of parturition, Parturition index – gestation index calculated.
- Offspring viability indicies: The standard unit of assessment was considered to be the litter, therefore values were first calculated for each litter and the group mean was calculated using their individual litter values. Group mean values included all litters reared to termination (Day 5 of age).

Implanatation losses – pre and post implanantation losses and impantation index, Live birth and viability indices – live birth index, viability index and delivery index, Sex ratio – sex ratio for surviving litter on Day 0, 1 and 4 post-partum and sex ratio at birth (total).

Statistics:

The following parameters were subjected to statistical analysis: haematology, blood chemistry and urinalysis, pre-coital intervals, gestation lengths, litter data- litter size, corpora lutea, implantation sites, litter weight, sex ratio, implantation losses, live birth index, viability indices, implantation index and delivery index, offspring bodyweight and bodyweight change, offspring surface righting, adult absolute and bodyweight relative organ weights.

The following statistical procedures were used: Data was assessed for dose response relationships by linear regression analysis, followed by one way analysis of variance (ANOVA) incorporating Levene's test for homogeneity of variance. Where variances were shown to be homogenous, pairwise comparissons were conducted using Dunett's test. In case of recovery group data, the analysis used was a two-tailed t-test incorporating Levene's test for homogeneity of variance. Where Levene's test showed unequal variances the data was analysed using non-parametric methods: Kruskal-Wallis ANOVA and Mann-Whitney 'U' test.

Non parametric methods were used to analyse implantation loss, offspring sex ratio and landmark developmental markers.

Probability values (p) are presented as follows: P<0.001***, P<0.01**, P<0.05*, P≥0.05 (not significant)

Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes;

1. Chi-squared analysis for differences in the incidence of lesions occuring with an overall frequency of 1 or greater. 2. Kruskal-Wallis one-way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions. Probability values (p) were calculated as follows: P<0.001 +++ --- *** P<0.01 ++ -- ** P<0.05 + - * P<0.1 (+) (-) (*) P≥0.1 (n.s.) +/- difference vs. control

Results and discussion

Results for P generation

Clincal signs:

Dehydration and staining around the ano-genital region was evident for one female treated at 300 mg/kg/day on Days 6 and 7. A second female showed dehydration on Day 7 and was hunched from Day 8-10. A third female showed staining of the ano-genital region on Day 7. Regression of these signs was evident thereafter.

Other signs in the 300 mg/kg/day consisted of increased salivation after dosing and up to one hour after dosing on occasion of animals of either sex during the treatment period. Staining around the mouth were recorded and instances of noisy respiration noted in 5 males and 1 recovery female. Regression of these signs was evident following cessation of treatment in recovery animals.

Increased salivation was observed in the 100 mg/kg/day group (both sexes) from week 3 with red/brown staining around the mouth observed. The incidence of signs was less in this group vs. the 300 mg/kg/day group. Increased salivation were detected up to 1 hour after dosing in the 30 mg/kg/day group (both sexes).

Mortality:

A 300 mg/kg/day female displayed signs of hunched posture, lethargy, laboured and gasping breathing and tiptoe gait. Termination on Day 6 and resulting pathology of this individual concluded that the death was not material toxicity related but rather the result of an inappropriate dosing technique.

Body weight and weight changes:

Males:

300 mg/kg/day - significant reduction through the test period vs. controls. Bodyweight gains were statistically higher in the recovery group during the treatment free period. Reduced bodyweight increases throught the treatment period inevitably resulted in significantly lower mean bodyweights from Day 15 onwards.

100 mg/kg/day - significantly reduced bodyweight gain during the first 3 weeks of treatment.

30 mg/kd/day - no adverse effects noted.

Females:

300 mg/kg/day - 1 individual showed substantial weight loss (28 g) in week 1. Four other individuals showed slight bodyweight losses, resulting in a significant reduction in mean bodyweight gain vs. controls in week 1.

100 mg/kg/day - significantly lower bodyweight gains in the first week of treatment vs. controls. Bodyweight gain during gestation was comparable to controls. Mean bodyweights on Day 0 and 4 of lactation were significantly lower vs. controls.

30 mg/kg/day - significant reduction in bodyweight on Day 0 and 4 post partum.

Food Consumption and compound intake:

Males:

300 mg/kg/day - significant reduction in consumption during week 1 in non recovery (-22 %) and recovery (-28 %) animals vs. controls. Reduced consumption was also noted in both groups in week 2. Intake was not measured during mating period however reduced intake was evident during this time. Reductions were evident in recovery animals through the remaining treatment period, although no difference was observed in the non-recovery group vs. controls. During the treatment-free period, treated animals intake was comparable to the controls.

100 mg/kg/day - significant reductions noted pre-mating when compared to controls. Intake improved and was comparable to controls after mating.

30 mg/kg/day - no adverse effects noted.

Females:

300 mg/kg/day - 25 % and 31 % reduction in intake during week 1 compared to controls in non-recovery and recovery groups. Recovery animals showed further reductions in intake up to week 5. Regression was evident during the treatment-free period.

100 mg/kg/day - 19 % reduction in week 1 and significant reductions through weeks 2 and 3 of the gestation period. Intake was comparable to controls during lactation.

30 mg/kg/day - Reduced intake during week 2 of gestation vs. controls.

Food efficiency:

Males:

300 mg/kg/day - reduced efficieny vs control through weeks 1 - 7. Increased in efficiency (vs control) during treatment-free period.

100 mg/kg/day - reduced efficiency vs control in week 1, although comparable to control though remainder of test.

30 mg/kg/day - no difference vs controls.

Females:

300 mg/kg/day - reduction in efficiency in week 1, comparable to controls through remainder of the test.

100 mg/kg/day - reduction in week 1, comparable to controls through remainder of the test.

30 mg/kg/day - reduction in week 1, comparable to controls through remainder of the test.

Water consumption and compound intake:

Males:

300 mg/kg/day - increased water intake noted in non-recovery individuals throught the whole test period versus control. Regression occuring in recovery males during treatment-free period.

100 mg/kg/day - increased water intake pre-mating (statistically) and post-mating (statistically, only in week 5) versus control.

30 mg/kd/day - increased water intake pre-mating (statistically) and post-mating (statistically, only in week 5) versus control.

Females:

300 mg/kg/day - significant increase during week 1 and 2 in the recovery females (week 1-3, for non-recovery females) versus controls. Recovery evident during the treatment-free period.

100 mg/kg/day - significant increase during week 1versus controls. Increase during gestation and early lactation , although not statistically significant.

30 mg/kg/day - not significantly different vs controls.

Haematological findings:

Males:

300 mg/kg/day - lower (not statistically significant) haemoglobin and erythrocyte values observed on Day 14. Significant (slight) reduction in reticulocyte counts versus controls on Day 14. At Day 42, significant reduction in haemoglobin and erythrocytes counts were oberved versus controls. Hematocrit counts also lower (although not significantly) at this timepoint. Regression of these findings was observed in the recovery males during the treatment-free period with the exception of erythrocyte counts. Significant increase in mean cell volume and mean cell haemoglobinwas observed in the recovery males versus controls.

30 and 100 mg/kg/day - No changes versus control.

Females:

300 mg/kg/day - no significant differences versus controls.

100 mg/kg/day - significant reduction in mean cell haemoglobin compared to controls on Day 14 (slight and in the absence of a dose-related response and other haemotological changes at this level, this finding was considered to have been incidental).

30 mg/kg/day - no significant change versus controls.

Clinical biochemistry findings:

Males:

300 mg/kg/day (pre-mating) - significant reduction in blood albumin on Day 14 (pre-mating). Lower A/G ratios and increased alanine aminotransferase levels were also evident, although not significantly so. Significant increase in blood urea levels versus controls. Significantly, blood cholesterol was reduced during pre-mating, versus control values.

300 mg/kg/day (pre-termination) - significant increase in blood urea levels versus controls with reduction in blood albumin levels evident also. Reduction of blood cholesterol and increase in alanine aminotrasferase continued at significant levels. Regression of changes observed during the treatment-free period in recovery males with slight reduction in plasma bilirubin noted, versus controls. Slight increases in A/G ratio and plasma chloride levels noted.

100 mg/kg/day - significant reduction in blood albumin on Day 14 (pre-mating) and Day 42. Reduction in blood choloesterol on Day 42 versus controls with an increse in alanine aminotransferase also noted.

30 mg/kg/day - reduced blood cholesterol pre-mating.

Females:

300 mg/kg/day (pre-mating) - significant reduction in blood albumin and A/G ratios on Day 14 (pre-mating). Significant increase in alanine aminotransferase levels and reduction in plasma chloride levels compared to controls observed. Significantly, blood cholesterol was reduced during pre-mating, versus control values although a dose response curve was not apparent.

300 mg/kg/day (pre-termination) - Day 4 post-partum blood levels were not significantly changed versus the controls.

30 and 100 mg/kg/day - reduced blood cholesterol pre-mating (significant).

Urinalysis findings:

No significant changes versus controls observed in any of the treated males.

Behaviour:

Noisy respiration noted in one female of 300 mg/kg/day group- also noted in clinical observations.

Functional observations:

No significant changes in treated animals versus controls.

Functional performance:

No significant changes in treated animals versus controls.

Sensory reactivity assessment:

No significant changes in treated animals versus controls.

Organ weight findings including organ / body weight ratios:

Males:

Individuals treated at 300 mg/kg/day had significant reductions in absolute epididymis weights versus controls, which reflected in lower bodyweight-relative epididymis weights. Lower absolute and bodyweight-relative testis weights were also evident at 300 mg/kg/day in comparison to the controls, althought only statistically significant for absolute weight. Elevated absolute adrenal weights versus controls, with statistically significant increase in bodyweight-relative adrenal weights observed in comparison to the controls. Elevation in bodyweight-relative liver weights were observed versus controls. Organ weight data for recovery males after the 14 treatment-free days still showed elevated bodyweight-relative adrenal weights when compared to controls. Bodyweight-relative spleen and thymus weights were also elevated when compared to controls. Bodyweight-relative brain weights were elevated compared to the controls. In the absence of histopathological correlates, these increases were not considered to represent delayed systemic toxicity.

No effects were noted in the 100 or 30 mg/kg/day treated individuals.

Females: No effects were detected in treated post partum females compared to controls.

Slight but statistically significant organ weight changes were evident for females treated at 100 and 30 mg/kg/day. These consisted of slight reduction in absolute heart weights (100 and 30 mg/kg/day). Higher bodyweight-relative brain weights were also observed for females treated at 100 and 30 mg/kg/day. A dose-related response was not evident and in the absence of histopathological changes in these organs, these findings were not considered to represent a true effect of treatment.

No significant organ weight changes were noted in the recovery females during the treatment-free period.

Histopathological findings:

Mammary gland

Tubuloalveolar differentiation of mammary tissue was seen in males treated at all three concentrations, although only statistically significant data was observed at 300 mg/kg/day. There was no evidence of regression of the observation in the recovery males after the treatment-free period. Minimal glandular hyperplasia of the mammary tissue was seen for four non-pregnant females treated at 300 mg/kg/day. This may have been an effect of treatment. Hyperplasia was not seen in recovery control or 300 mg/kg/day females following completion of the treatment free period.

Ovaries

Follicular cysts were seen among non-pregnant females in the 300 mg/kg/day group, this may have been an effect in the absence of directly comparable controls. Follicular cysts were seen in the recovery females versus controls suggesting that the effect had not regressed.

Testes

Leydig cell atrophy was seen in relation to treatment for males treated with 300 mg/kg/day and at 100 mg/kg/day, although not statistically significant at this level. The condition regressed among the recovery males after the treatment free period. Moderate or severe testicular atrophy was seen for two recovery males. This condition does occur spontaneously among laboratory maintained rats and there was no evidence to suggest this was a treatment related consequence.

Seminal vesicles/ coagulating gland

Reduced secretory content as indicated by smaller organ size was seen in relation to treatment for males treated with 300 mg/kg/day and 100 mg/kg/day compared to control, but not statistically significant at 30 mg/kg/day. There was no evidence of regression of the condition among 300 mg/kg/day males after the treatment-free period has elapsed.

Prostate

Reduced secretory content as indicated by smaller organ size was seen in relation to treatment for males treated with 300 mg/kg/day and 100 mg/kg/day, but not at 30 mg/kg/day. There was no convincing regression observed in the recovery males.

Liver

Centrilobular hepatocyte enlargement was seen in relation to treatment for males treated with 300 and 100 mg/kg/day with the effects also evident at 30 mg/kg/day (statistically significant). Females were also affected at 300 mg/kg/day at (not statistically significant) and 100 mg/kg/day (statistically significant). Regression was observed in both sexes in the recovery animals.

Kidneys

A greater incidence of higher grades of severity of groups of basophillic tubules and tubular dilatation were seen as a consequence of treatment for males with 300 mg/kg/day compared to controls (statistically significant)) but not at other dose levels. Not observed (convincingly) for females. Both conditions regressed in the recovery group.

Adrenal glands

Cortical vacuolation is relatively common in lab-maintained rats and is especially prevalent among males and more rarely seen among females. The condition was significantly less prevalent among males treated at 300 mg/kg/day and 100 mg/kg/day. Although this may be a spurious group distribution of incidence and severity grades and effect of treatment on the adrenal cortex cannot be excluded. A similar effect was not seen in the females. A group differential was maintained among recovery animals suggesting that any effec was not fully regressed.

Lungs

Groups of alveolar macrophages were prevalent among control animals of either sex and grades of severity ranged from minimal to moderate. Such a macrophage response was rather greater than might normally be seen in te control animals of this age. The incidence and severity of alveolar macrophage populations was significantly lower for males and femlaes treated at 300 mg/kg/day (stat. analysis not performed on females) and 100 mg/kg/day. Although such incidence and severity could be fortuitous, an effect of treatment cannot be excluded. No evidence of alveolar macrophage accumulation after the treatment-free period had elapsed, suggesting regression of any effect.

Pituitary

Vacuolation of pars anterior cells is commonly seen among male rats but it is rarely present in female rats of this age. The prevalence and severity grades of vacuolation were normal or slightly above normal for control males but significantly lower for males treated at 300 mg/kg/day, indicating a dose-related effect. This effect was not seen in females or males treated at other concentrations. There was no evidence of regression in the recovery males.

Uterus/ cervix

Dilation of the uterine horn, with or without keratinisation in the cervix was found in one female treated with 30 mg/kg/day and one female treated with 100 mg/kg/day which displayed in utero total litter loss and one non-pregnant female treated with 30 mg/kg/day, 2 non-pregnant females treated with 100 mg/kg/day and 2 nonpregnant females from the 300 mg/kg/day dose groups. This simply represents normal cyclical changes in the female rat. In addition, necrotic contents were present in the uterus from one female treated at 100 mg/kg/day. This female showed a corpus luteum and implantation site during the post mortum procedure, therefore this was considered to represent resorption of the foetuses.

Vagina

Hyperplasia of the vaginal epithelium was seen for 4 non-pregnant females at 300 mg/kg/day, but allowing for cyclical changes, there was insufficient evidence to suggest an effect of treatment. Similarly, higher grades of severity of vascuolar degeneration of the post partum vaginal epithelium as normal conversion from mucinous to non-mucinous morpholgy were seen among intermediate dose females compared to

controls. There was no convincing effect of the treatment in this study. Keratinisation of the vaginal epithelium is a normal cyclical change in the female rat.

Reproductive function / performance (P0):

One female treated with 300 mg/kg/day showed a continuous anestrus interval and also failed to mate. Another female treated with 300 mg/kg/day showed extended oestrus. This female mated but did not achieve pregnancy. These events were considered unusual.

Reproductive performance:

There were no pregnant females observed at 300 mg/kg/day. Seven pregnant females were evident at 100 mg/kg/day and three females at this dose level which showed positive evidence of mating but did not achieve pregnancy. One female treated with 100 mg/kg/day showed evidence of mating and post-mortem examinations revealed the presence of a corpus luteum and an implantation site, however, this female did not produce a live litter. All females treated with 30 mg/kg/day showed positive evidence of mating, although two females did not achieve pregnancy. One female treated at this dose level did not deliver a live litter but showed two dead foetuses in utero during the post-mortem procedure. Pregnancy was achieved in the eleven control females which showed positive evidence of mating.

Results for F1 generation

General toxicity

Clinical signs:

Daily clinical observations of offspring did not reveal any clinical signs considered to be related to test material toxicity. The clinical signs observed were those commonly observed in offspring in reproductive studies of this type, and were not considered to represent adverse effects of treatment.

Mortality:

For interim death offspring, macroscopic findings were confined to autolytic changes, with the exception of three offspring from one 30 mg/kg/day litter, which were cannibalised. These findings are commonly observed in interim death offspring in reproductive studies of this type, and were considered not to represent an effect of treatment.

Body weight and weight changes:

A slight but statistically significant reduction in Day 4 post partum litter weights was evident for 30 mg/kg/day litters when compared to controls (P<0.05). The significance achieved was minimal and in the absence of a dose-related response, this isolated intergroup difference was considered to have arisen incidentally and was of no toxicological importance.

Developmental neurotoxicity (F1):

Surface righting assessments on Day 1 post partum did not reveal any significant intergroup differences between litters from treated animals when compared to litters from controls.

Table 1: Average body weight and body weight gains during 56 days of treatment (P0)

Dose rate (mg/kg/day)	Male Body Weights at Day (g)									Total Weight Gain (g)
	1	8	15	22	29	36	43	50	57	
Male										
Control	340	370	390	412	436	456	461	-	-	120
Low	342	370	393	411	437	455	466	-	-	124
Mid	340	359	375	395	421	439	449	-	-	108
High	343	356	371	384	404	418	425	-	-	83*
Recovery control	346	387	422	455	484	507	528	548	556	210
Recovery high	344	352	367*	382*	395*	406*	412*	445*	463*	120**
Female Body Weights (Weight Gain) During (g);										
Dose rate (mg/kg/day)	Maturation (at day)			Gestation (at day)				Lactation (at day)		Total Weight Gain (g)
	1	8	15	0	7	14	20	0	4	
Control	241	250 (9)	256 (6)	271	304 (33)	340 (36)	421 (81)	324	332 (8)	-
Low	238	241 (3)	246 (5)	253	284 (31)	314 (30)	377* (63)	301*	300** (-1)	-
Mid	233	233 (1**)	239 (6*)	248	282 (34)	312 (31)	382 (70)	292*	301* (9)	-
High	242	240 (-2**)	250 (9)	-	-	-	-	-	-	-
Recovery control	236	245	254	262	271	280	283	290	295	59
Recovery high	236	235	242	249	257	263	267	279	279	43

* Significantly different (p <0.05) from the control.

** Significantly different (p <0.01) from the control.

*** Significantly different (p <0.001) from the control.

Table 2: Selected haematology, clinical chemistry and pathological findings

Doses (mg/kg/day)	Control	30	100	300	Control	30	100	300
	male				female			
Number of animals/group	5	5	5	5	5	5	5	5 (day 56 recovery)
Haematology(day 42 for males and day 4 post-partum for females)								
- Hb (g/dl)	16.6	16.2	16.1	15.2**	13.3	12.7	12.4	15.5
- RBC (10^{12}/L)	8.89	8.78	8.46	8.09**	6.91	6.62	6.53	8.29
- Hct (%)	47.5	46.9	46.9	44.9	39.6	37.4	36.9	45.4
- MCH (pg)	18.7	18.5	19.0	18.7	19.4	19.1	19.0	18.7
- MCV (fl)	53	53	55	55	57	56	57	55
- MCHC (g/df)	34.9	34.7	34.2	33.8	33.7	33.8	33.6	34.1
- WBC (10^9/L)	10.8	9.2	11.3	8.0	9.0	7.5	12.1	7.3
Blood chemistry(day 42 and day 4 post-partum for females)								
- Urea (mg/dl)	29	32	29	37**	35	40	50	44
- Glucose (mg/dl)	147	159	140	153	118	122	128	163
- Tot. Prot. (g/dl)	6.57	6.45	6.34	6.42	5.65	5.74	5.57	7.58
- Albumin (g/dl)	3.6	3.5	3.3*	3.3**	3.2	3.2	3.1	4.2
- A/G ratio	1.21	1.17	1.14	1.09	1.34	1.30	1.20	1.26
- Na+ (mmol/L)	150	150	150	150	151	149	152	152
- K+ (mmol/L)	4.58	4.32	4.46	4.16	4.80	4.08	4.59	4.20
- Cl- (mmol/L)	104	103	105	104	106	105	104	107
Pathology		male				female		
Number of animals/group	12	12	12	12	12	12	12	11[a]
- External,mass under right forelimb	0	0	1	0	0	0	0	0
- Internal,epididymides: small	0	0	1	0	0	0	0	0
- Internal,right kidney: hydronephrosis	0	0	1	0	0	0	0	0
- Internal,lungs: mottled appearance	0	0	1	0	0	0	0	0
- Internal,mandibular lymph nodes: enlarged	0	0	1	0	0	0	0	0
- Internal,mass: approx.. 2 cm, spherical, containing green/yellow substance	0	0	1	0	0	0	0	0
- Internal,seminal vesicles: small	0	0	0	4	-	-	-	-
- Internal,prostate: small	0	0	0	4	-	-	-	-
- Internal,testes: small	0	0	1	0	-	-	-	-
- Internal,adrenals: pale	0	0	0	0	0	1	0	0
- Internal,cervical lymph nodes: enlarged	0	0	0	0	0	0	0	1
- Internal,lungs: reddened	0	0	0	0	1	0	0	1
- Internal,mass: approx. 1.5 cm, containing white coloured viscous liquid	0	0	0	0	0	0	0	1
- Internal,ovaries: dark red discolouration	-	-	-	-	0	0	0	1
- Internal,stomach: sloughing- glandular region	0	0	0	0	0	1	0	0
- Internal,Uterus: 1 dead foetus in each horn	-	-	-	-	0	1	0	0
- No abnormalities	12	12	9	8	11	11	12	9

Table 3: Absolute and relative organ weights (P0)

		Males (non-recovery)				Females (non-recovery)			Females (recovery)	
DAILY DOSE (mg/kg bw/day)		Control	30	100	300	Control	30	100	Control	300
NUMBER OF ANIMALS		12	12	12	12	7-11	7-11	7-11	5	4-5
BODY WEIGHT (g)[a]		461	466	449	425	332	301	304	295	279
BRAIN										
Absolute Weight[a]	g	2.0179	2.0543	2.0266	2.0070	1.9028	1.8502	1.9078	1.9121	1.8922
Per Body Weight[a]	%	0.4423	0.4419	0.4535	0.4754	0.5755	0.6164*	0.6282*	0.6530	0.6810
ADRENALS										
Absolute Weight[a]	g	0.0562	0.0562	0.0527	0.0636	0.0760	0.0705	0.0700	0.0628	0.0571
Per Body Weight[a]	%	0.0122	0.0121	0.0118	0.0150**	0.0229	0.0234	0.0229	0.0214	0.0202
EPIDIDYMIDES										
Absolute Weight[a]	g	1.2917	1.3495	1.2100	1.0385***	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
Per Body Weight[a]	%	0.2824	0.2900	0.2695	0.2448**	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
HEART										
Absolute Weight[a]	g	1.7237	1.6602	1.5566	1.5537	1.3490	1.1218*	1.1404*	1.0731	1.1116
Per Body Weight[a]	%	0.3738	0.3563	0.3477	0.3666	0.4053	0.3726	0.3762	0.3661	0.3994
KIDNEYS										
Absolute Weight[a]	g	3.1949	3.2058	3.0922	3.1681	1.9178	1.8878	1.9243	1.9781	1.8940
Per Body Weight[a]	%	0.6952	0.6877	0.6910	0.7431	0.5778	0.6261	0.6316	0.6713	0.6802
LIVER										
Absolute Weight[a]	g	15.6764	15.5205	14.9837	15.8695	12.7095	11.6779	11.9166	9.3369	9.3088
Per Body Weight[a]	%	3.3934	3.3273	3.3404	3.7250*	3.8193	3.8884	3.9040	3.1752	3.3435
SPLEEN										
Absolute Weight[a]	g	0.7653	0.7845	0.7309	0.6956	0.6289	0.5669	0.5916	0.5168	0.4972
Per Body Weight[a]	%	0.1659	0.1688	0.1634	0.1638	0.1888	0.1890	0.1941	0.1746	0.1784
TESTES										
Absolute Weight[a]	g	3.4920	3.5732	3.2223	3.1171*	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
Per Body Weight[a]	%	0.7641	0.7701	0.7177	0.7350	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]
THYROID										
Absolute Weight[a]	g	0.0178	0.0189	0.0196	0.0170	0.0149	0.0118	0.0135	0.0141	0.0127
Per Body Weight[a]	%	0.0039	0.0041	0.0043	0.0040	0.0045	0.0040	0.0044	0.0049	0.0046
THYMUS										
Absolute Weight[a]	g	0.3756	0.3925	0.3732	0.3393	0.2620	0.2118	0.2171	0.3301	0.2999
Per Body Weight[a]	%	0.0823	0.0838	0.0840	0.0801	0.0790	0.0706	0.0715	0.1118	0.1072
OVARIES										
Absolute Weight[a]	g	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	0.2076	0.0935	0.0958	0.0851	0.0712
Per Body Weight[a]	%	n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	0.0594	0.0312	0.0313	0.0289	0.0256
UTERUS										
Absolute Weight[a]		n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	0.8779	0.7427	0.8500	0.9308	0.6539
Per Body Weight[a]		n.a.[b]	n.a.[b]	n.a.[b]	n.a.[b]	0.2642	0.2484	0.2787	0.3140	0.2356

Table 4: Number of male animals with histopathological findings in reproductive-related organs. Incidence in percent in parenthesis

Dose levels (mg/kg/day)	**0**	**30**	**100**	**300**	**Recovery 0**	**Recovery 300**
No. of animals	n = 12	n = 12	n = 12	n = 12	n= 5	n= 5
Mammary gland - Tubuloalveolar differentiation						
No section	2	2	2	3	0	0
Absent	7	4	3	1	5	1
Minimal	3 (25%)	4 (33%)	6 (50%)	2 (17%)	0	0
Slight	0	2 (17%)	1 (8%)	4 (33%)	0	1 (20%)
Moderate	0	0	0	2 (17%)	0	3 (60%)
Prostate - Reduced secretory content						
No section	0	0	1	0	0	0
Absent	23	11	7	4	5	1
Present	0	1 (8%)	4 (33%)	8 (67%)	0	4 (80%)
Prostate - Chronic inflammatory cell foci						
Absent	12	12	11	11	5	5
Slight	0	0	0	1 (8%)	0	0
Seminal vesicles – Reduced secretory content						
Vesicle 1						
No section	0	0	1	0	0	0
Absent	11	10	5	0	5	2
Present	1 (8%)	2 (16%)	6 (50%)	12 (100%)	0	3 (60%)
Vesicle 2						
No section	0	0	1	0	5	5
Absent	11	10	6	0	0	0
Present	1 (8%)	2 (16%)	5 (42%)	12 (100%)	0	0
Testes - Atrophy						
Testis 1						
Absent	12	12	12	12	5	3
Moderate	0	0	0	0	0	1 (20%)
Severe	0	0	0	0	0	1 (20%)
Testis 2						

Dose levels (mg/kg/day)	**0**	**30**	**100**	**300**	**Recovery 0**	**Recovery 300**
Absent	12	12	12	12	5	4
Severe	0	0	0	0	0	1 (20%)
Leydig cell						
Absent	11	12	9	1	5	4
Present	1 (8%)	0	3 (25%)	11 (92%)	0	1 (20%)

Table 5: Number of female animals with histopathological findings in reproductive-related organs, only females that failed to mate/non-pregnant. Incidence in percent in parenthesis.

Dose levels (mg/kg/day)	**0**	**30**	**100**	**300**
No. of animals	n=1	n=2	n= 4	n= 11
No. animals that failed to mate	1 of 12 (8%)	0	1 of 12 (8%)	1 of 11 (9%)
No. of animals not pregnant	0	2 of 12 (16%)	3 of 12 (25%)	10 of 11 (90%)
Mammary gland				
Glandular hyperplasia (minimal)	0	0	0	4 of 11 (36%)
Ovaries				
Cystic corpora lutea	0	1 of 2 (50%)	1 of 4 (25%)	3 of 11 (27%)
Follicular/fluid-filled cyst	0	0	2 of 4 (50%)	9 of 11 (82%)
Haemorrhagic cyst	0	0	0	1 of 11 (9%)
Vacuolation stroma	0	0	0	2 of 11 (18%)
Thyroid				
Follicular cell hypertrophy (minimal)	0	1 of 2 (50%)	0	5 of 11 (45%)
Uterus/Cervix				
Dilatation horn 1				
Minimal	0	0	1 of 4 (25%)	1 of 11 (9%)
Slight	0	1 of 2 (50%)	1 of 4 (25%)	1 of 11 (9%)
Dilatation horn 2				
Minimal	0	0	2 of 4 (50%)	1 of 11 (9%)
Slight	0	1 of 2 (50%)	0	1 of 11 (9%)
Endometrial gland proliferation	0	0	0	1 of 11 (9%)
Keratinisation cervix	0	2 of 2 (100%)	3 of 4 (75%)	1 of 11 (9%)
Vagina				
Epithelial hyperplasia				
Minimal	0	0	0	4 of 11 (36%)
Epithelial keratinisation	0	2 of 2 (100%)	1 of 4 (25%)	2 of 11 (18%)
Keratin cyst	0	0	0	1 of 11 (9%)

Table 6: Number of female animals with histopathological findings in reproductive-related organs, only pregnant/recovery females. Incidence in percent in parenthesis.

Dose levels (mg/kg/day)	**0**	**30**	**100**	**300**	**Recovery 0**	**Recovery 300**
No. of females	n=11	n=9	n=7	n=0	n=5	n=5
Ovaries						
Follicular/fluid-filled cyst						
Absent	9	7	4		5	1
Present	2 (18%)	2 (22%)	3 (43%)		0	4 (80%)
Cystic corpora lutea						
Absent	6	7	6		5	5
Present	5 (45%)	2 (22%)	1 (14%)		0	0
Vagina						
Epithelial keratinisation						
No section	0	1	0		0	1
Absent	11	8	7		2	2
Present	0	0	0		3 (60%)	2 (50%)

Table 7: Mating performance, Fertility, Gestation Length (P0)

Dose Level (mg/kg/day)	Number of males paired	Number of females			Pre-coital interval (Days)						
		Paired	Mated	Pregnant	1	2	3	4	5	6	14
0 (control)	12	12	11	11	1	2	4	3	0	0	1
30	12	12	12	10	5	1	3	3	0	0	0
100	12	12	11	8	4	1	3	1	1	0	0
300	12	11	10	0	3	3	0	0	1	2	1

Dose level (mg/kg/day)	Mating index (%)	Fertility index (%)	Gestation Length (Days)						Females with live offspring	Gestation Index (%)
			22	22.5	23	23.5	24	Not confirmed		
0 (control)	91.7	100	2	2	3	4	0	0	11	100
30	100.0	83.3	1	2	3	2	1	0	9	90
100	91.7	63.6	0	1	5	0	0	1	7	87.5
300	90.9	0	-						-	-

Table 8: Litter and offspring bodyweight data

Dose group (mg/kg/day)		Number of corpora lutea	Number of implantation sites	Total number of offspring born	Number of live offspring		Litter weight (g)		Offspring weight (g)				Offspring bodyweight change (g)	
					Day 0	Day 4	Day 0	Day 4	Day 0		Day 4		Days 0 - 4	
									♂	♀	♂	♀	♂	♀
0 (control)	mean	16.7	14.1	13.1	13.0	12.8	85.5	118.1	6.9	6.5	9.8	9.2	2.9	2.7
	sd	3.6	1.9	2.5	2.6	2.7	11.4	12.8	0.8	0.8	1.5	1.7	0.8	1.0
	n	11	10	11	11	11	11	11	11	11	11	11	11	11
30	mean	14.7	12.1	10.9	10.2	10.0	68.6	97.7*	7.1	6.6	10.5	9.6	3.4	3.0
	sd	5.8	3.8	3.3	2.6	2.5	11.2	12.4	0.8	0.7	1.8	1.4	1.0	0.8
	n	9	7	9	9	9	9	9	9	9	9	9	9	9
100	mean	14.0	10.4	11.7	11.3	10.9	76.7	100.6	7.2	6.6	10.0	9.3	2.8	2.7
	sd	7.8	4.8	3.4	3.7	3.5	23.3	22.4	0.9	0.7	1.8	1.5	1.0	1.1
	n	7	7	7	7	7	7	7	7	7	7	7	7	7
300	mean													
	sd													
	n													

Table 9: Implantation losses and offspring survival indices

Dose Group (mg/kg/day)		Pre-Implantation Loss (%)	Post-Implantation Loss (%)	Live Birth (%)	Viability Index (%)
0 (control)	mean	11.9	9.5	99.1	98.4
	sd	13.2	10.3	3.0	3.7
	n	10	10	11	11
30	mean	10.6	7.8	95.8	98.0
	sd	18.2	7.6	12.5	4.1
	n	7	7	9	9
100	mean	18.7	22.9	95.6	96.3
	sd	16.8	35.5	8.5	6.4
	n	7	7	7	7
300	mean				
	sd				
	n				

Table 10: Sex ratio – Group mean litter values

Dose Group (mg/kg/day)		Sex Ratio (% Males) on (Post Partum) Day;		
		At Birth	1	4
0 (control)	mean	47.5	47.9	48.8
	sd	9.0	8.7	9.4
	n	11	11	11
30	mean	45.4	45.2	44.7
	sd	14.9	14.7	14.7
	n	9	9	9
100	mean	48.7	48.0	47.9
	sd	10.1	10.9	10.9
	n	7	7	7
300	mean	-	-	-
	sd			
	n			

Dose Group (mg/kg/day)	Number of Litters	Sex Ratio (Fraction of Males) on (Post Partum) Day;		
		At Birth	1	4
0 (control)	11	0.48	0.48	0.49
30	10	0.44	0.43	0.43
100	12	0.48	0.46	0.46
300	0	-	-	-

Table 11: Offspring clinical observations – Summary Incidences

Dose Level (mg/kg/day)	Number of Litters	Clinical Observation	Number of Offspring (Number of Litters) Affected (Post Partum) Day;					
			0	1	2	3	4	5
0 (control)	11	Abdominal bruising	0	0	0	1F(1)	0	0
		Bruised dorsal surface	1M(1)	1M(1)	0	0	0	0
		Found dead	1F(1)	0	0	0	0	2F(2)
		No milk present in stomach	1F(1)	1F(1)	1F(1)	2F(2)	3F(3)	0
		Missing	0	0	1F(1)	0	1F(1)	1M(1)
		Missing tail	1F(1)	1F(1)	1F(1)	1F(1)	1F(1)	1F(1)
		Pale	1F(1)	1F(1)	1F(1)	1F(1)	1F(1)	0
		Physical injury to ventral surface	1F(1)	1F(1)	1F(1)	1F(1)	0	0
		Scab formation on ventral surface	1F(1)	1F(1)	1F(1)	1F(1)	0	0
		Small	1F(1)	1F(1)	1F(1)	1F(1)	3F(3)	0
		Swollen left hindlimb	0	0	1M(1)	0	0	0
		Swollen right forelimb	0	0	1M(1)	1M(1)	0	0
		No abnormalities	(6)	(7)	(5)	(6)	(7)	(8)
30	9	Bruised snout	1F(1)	0	0	0	0	0
		Cannibalised	3(1)	0	0	0	0	0
		Found dead	1F, 2M(1)	0	0	0	0	0
		No milk present in stomach	0	0	0	1F(1)	0	0
		Missing	0	0	1M(1)	1F(1)	0	0
		Pale	0	1M(1)	0	0	0	0
		Small	1F(1)	1F(1)	1F(1)	1F(1)	1F(1)	1F(1)
		No abnormalities	(6)	(7)	(7)	(7)	(8)	(8)
100	7	Bruised snout	1F(1)	0	0	0	0	0
		Cold	1F, 2M(1)	0	0	0	1F(1)	0
		Found dead	1F, 2M(2)	0	0	0	0	1F(1)
		Missing	0	1M(1)	0	0	2M(1)	1F(1)
		No milk present in stomach	1F(1)	0	1F(1)	1F(1)	1F(1)	0
		Pale	1M(1)	1M(1)	1M(1)	0	0	0
		Small	2F(2)	2F(2)	2F(2)	1F(1)	9F, 7M (3)	8F, 7M (2)
		Swollen right hindlimb	0	0	1F(1)	1F(1)	1F(1)	0
		Weak	0	0	0	0	1F(1)	0
		No abnormalities detected	(4)	(5)	(4)	(4)	(3)	(4)
300	0	-	-	-	-	-	-	-

Table 12: Reflexological responses for offspring – Group mean litter values

Dose level (mg/kg/day)		Surface Righting Reflex (% passed)
0 (control)	mean	91.2
	sd	7.4
	n	11
30	mean	90.9
	sd	11.4
	n	9
100	mean	90.3
	sd	13.3
	n	7
300	mean	-
	sd	-
	n	-

Table 13: Necropsy findings of offspring – group incidences

Observation	Number of Offspring (Litters) Affected at Dose Level (mg/kg/day)			
	0 (control)	30	100	300
INTERIM DEATHS				
Number of offspring	3F(3)	2M, 1F, 3U(1)	2M, 2F(3)	-
Autolytic changes detected	2F(2)	2M, 1F(1)	2M, 2F(3)	-
Cannibalised	0	3U(1)	0	-
No abnormalities detected	1F(1)	0	0	-
TERMINAL KILL				
Missing tail	1F(1)	0	0	-
Scab formation on ventral surface	1F(1)	0	0	-
Small	0	1F(1)	2F(2)	-
LITTER WITH NO ABNORMALITIES	9	8	5	-

3.11 Specific target organ toxicity – single exposure

Not evaluated in this CLH proposal.

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 Study 1

Study reference:

Takaaki Umano, Ryota Tanaka, Kanji Yamasaki, Endocrine-mediated effects of 4,4'-(hexafluoroisopropylidene)diphenol in SD rats, based on a subacute oral toxicity study
Arch Toxicol (2012) 86:151–157.

Test type:

The study was conducted according to OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents), GLP-compliant.

Test substance:

- Test material used in the study is Bisphenol AF (BPAF, EC nr: 216-036-7, CAS no: 1478-61-1).

Test animals:

- Rat/Sprague-Dawley/male/female
- 10 animals per sex per dose
- Age and weight at the study initiation: 8 weeks and ca 310-340 g (males) and ca 203-226 g (females).

Administration/exposure:

- Route of administration – oral (gavage)
- Duration and frequency of test/exposure period: daily for 28 days.

- Doses/concentration levels: 10, 30 and 100 mg/kg bw/day. Dose selection rationale: Chosen after completition of a range-finder test. Concentrations did not induce death or severe suffering.
- Control group and treatment: yes, concurrent vehicle
- Vehicle: olive oil - OECD recommended to aid solubility of test item.
- Amount of vehicle: 5 mL/kg/bw

Statistical methods:

Bartlett's variance test was performed for the parametric data. Bartlett's test revealed a homogeneous variance, so one-way analysis of variance was conducted and if the result of the one-way analysis was significant, Dunnett's test was performed to compare the treated and the control groups. Data with an inhomogeneous variance shown by Bartlett's test or nonparametric data were subjected to the Kruskal–Wallis rank test, and if a significant divergence was observed, a Dunnett's approach was carried out. Incidence rates of abnormal estrous cycles, gross pathological findings, and histopathological findings were analyzed by Fisher's "exact" probability test. In the evaluation of the examination results, when a divergence from the control was found at a significance level of 1 or 5%, it was regarded as a significant change.

A male rat in the control group was diagnosed as being the subject of an administration error on gross and histopathological examination. The error was thought to have happened just before day 22, based on changes in body weights and food consumption, so data that contained body weights at days 26 and 28, food consumption at days 22 and 28, and all organ weights of this rat were excluded from the statistical analysis.

Results and discussion:

Sensory activity, grip strength and motor activity assessments: not assessed
Ophthalmologic findings: incidence and severity: not assessed
Haematological findings: incidence and severity: not assessed

Endocrine disrupting potential:
Positive.

Clinical signs:
Several male and female rats in the 100 mg/kg group showed salivation from the first week, and this sign disappeared within 90 min of administration. No other abnormal general findings were observed in any of the groups.

Salivation in the 100 mg/kg group was not found to be a sign of toxicity because this sign disappeared soon after administration and no other related changes were observed.

Mortality:
No mortality observed.

Food consumption and compound intake:
A decrease in body weight gains was found in the male rat 100 mg/kg group and the female rat 30 and 100 mg/kg groups from the first week after administration and was accompanied by decreased food consumption.

Water consumption and compound intake:
In male rats, white blood cell counts, total cholesterol, and albumin values decreased in the 100 mg/kg group. In female rats, cholinesterase and total cholesterol values decreased and total bilirubin values increased in the 100 mg/kg group.
Serum T4 values increased in the 100 mg/kg groups of both sexes, but no changes in TSH were detected in any treated groups.

Organ weight findings including organ / body weight ratios:
In males, the relative weights of kidney, adrenal, and brain increased signifcantly in the 100 mg/kg group and the absolute weights of prostate, ventral prostate, seminal vesicle, liver, heart, and spleen decreased in this group. In female rats, the relative brain weights increased significantly in the 30 and 100 mg/kg groups, and the absolute heart weights decreased in the 100 mg/kg group.

Gross pathological findings:
Dilatation of the large intestinal lumen was observed in 9 male and female rats in the 100 mg/kg groups, respectively.

Histopathological findings:
In the male rats, the incidence of changes, such as atrophy of testicular Leydig cells, hypertrophy of the adrenal zona fasciculata, and decreased hepatocytic glycogen, was higher in the 100 mg/kg group than in the control group. In addition, decreased hematopoiesis in the bone marrow and spleen, atrophy of the mammary glands, and atrophy of pituitary basophilic cells were also observed in the 100 mg/kg group. In female rats, hypertrophy of the adrenal zona fasciculata and decreased hepatocytic glycogen were detected in several rats given the chemical.

Table 14: Mean body weight changes following 28 days of treatment

ose Group	Mean Initial Body Weight (g)		Mean Terminal Body Weight (g)	
	Male	Female	Male	Female
Control	324 ± 14	211 ± 7	449 ± 27	274 ± 18
10 mg/kg/day	324 ± 13	215 ± 11	451 ± 26	277 ± 18
30 mg/kg/day	325 ± 15	213 ± 10	450 ± 33	255 ± 18*
100 mg/kg/day	326 ± 13	214 ± 11	396 ± 29**	253 ± 15*

* statistically different from control group ($p < 0.05$)

** statistically different from control group ($p < 0.01$)

Table 15: Selected haematology, clinical chemistry and pathological findings.

aily Dose	Male				Female			
(mg/kg bw/day)	Control	10	30	100	Control	10	30	100
No. of animals	10	10	10	10	10	10	10	10
Haematology								
- WBC (x10³/mm³)	12.28 ± 1.05	11.83 ± 2.66	11.17 ± 2.67	9.17 ± 2.46*	8.59 ± 2.49	9.06 ± 2.15	9.28 ± 1.77	8.90 ± 1.84
Blood chemistry								
- Cholinesterase (IU/L)	54 ±18	56 ± 19	48 ± 11	50 ± 10	525 ± 163	537 ± 98	465 ± 236	300 ± 127*
- Total cholesterol (mg/dL)	69 ± 8	60 ± 12	60 ± 9	49 ± 7**	69 ± 12	65 ± 7	60 ± 11	47 ± 5**
- Total bilirubin (mg/dL)	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.04 ± 0.02**
- Albumin (g/dL)	3.00 ± 0.28	3.05 ± 0.12	3.05 ± 0.09	2.84 ± 0.11**	3.51 ± 0.45	3.56 ±0.16	3.35 ± 0.33	3.30 ± 0.27
- T4 (ng/dL)	3.704 ± 0.452	4.158 ± 0.826	4.061 ± 0.846	4.754 ± 0.762**	2.397 ± 0.297	2.376 ± 0.443	2.826 ± 0.950	3.671 ± 0.479**
Histopathology								
- Bone marrow,decreased hematopoiesis	0	0	0	4	0	NE	NE	0
- Spleen,decreased extramedullary	0	0	0	2	0	NE	NE	0
- Testis,atrophy of Leydig cells	0	0	0	5*				
- Mammary gland,atrophy of glands	0	0	0	3	0	NE	NE	0
- Adrenal gland,hypertrophy of zona fasiculata	1	1	0	8**	0	1	1	2
- Pituitary gland,atrophy of basophilic cells	0	0	0	1	0	NE	NE	0
- Liver,decreased hepatocytic glycogen	1	0	1	8**	0	0	1	2
- Organs in thoracic cavity,inflammation and granuloma	1	NE	NE	0	0	NE	NE	0

NE not examined

* significant difference from control ($p < 0.05$)

** significant difference from control ($p < 0.01$)

Table 16: Absolute and relative organ weights

Daily Dose	Male				Female			
(mg/kg/day)	Control	10	30	100	Control	10	30	100
No. of animals	9	10	10	10	10	10	10	10
Organ Weights								
Prostate (ventral and dorsolateral)								
mg	1068 ± 188	1134 ± 179	1061 ± 189	827 ± 183*	-	-	-	-
g/100 g	0.237 ± 0.045	0.251 ± 0.031	0.236 ± 0.041	0.208 ± 0.039	-	-	-	-
Ventral prostate								
mg	631 ± 166	741 ± 110	676 ± 150	474 ± 112*	-	-	-	-
g/100 g	0.139 ± 0.034	0.164 ± 0.021	0.150 ± 0.030	0.120 ± 0.027	-	-	-	-
Seminal vesicle								
g	1.41 ± 0.19	1.43 ± 0.30	1.38 ± 0.24	1.02 ± 0.36*	-	-	-	-
g/100 g	0.312 ± 0.045	0.317 ± 0.069	0.307 ± 0.045	0.254 ± 0.079	-	-	-	-
Liver								
g	17.13 ± 2.18	16.90 ± 1.37	16.38 ± 2.85	14.10 ± 1.31**	-	-	-	-
g/100 g	3.778 ± 0.322	3.747 ± 0.179	3.623 ± 0.399	3.564 ± 0.271	-	-	-	-
Kidney								
g	3.02 ± 0.23	3.01 ± 0.18	3.00 ± 0.37	2.89 ± 0.30	-	-	-	-
g/100 g	0.667 ± 0.021	0.671 ± 0.058	0.666 ± 0.064	0.729 ± 0.043*	-	-	-	-
Heart								
g	1.28 ± 0.09	1.32 ± 0.08	1.28 ± 0.09	1.13 ± 0.13**	0.87 ± 0.08	0.88 ± 0.05	0.82 ± 0.11	0.78 ± 0.06*
g/100 g	0.283 ± 0.013	0.293 ± 0.018	0.285 ± 0.011	0.286 ± 0.017	0.316 ± 0.015	0.319 ± 0.021	0.321 ± 0.024	0.308 ± 0.020
Spleen								
g	0.71 ± 0.08	0.70 ± 0.08	0.71 ± 0.10	0.59 ± 0.09*	-	-	-	-
g/100 g	0.158 ± 0.018	0.155 ± 0.015	0.158 ± 0.020	0.149 ± 0.016	-	-	-	-
Adrenals								
mg	58 ± 8	58 ± 11	56 ± 4	63 ± 9	-	-	-	-
g/100 g	0.013 ± 0.002	0.013 ± 0.002	0.012 ± 0.001	0.016 ± 0.003**	-	-	-	-
Brain								
g	2.17 ± 0.05	2.21 ± 0.09	2.23 ± 0.06	2.19 ± 0.07	2.03 ± 0.05	2.00 ± 0.06	2.05 ± 0.10	2.02 ± 0.08
g/100 g	0.482 ± 0.034	0491 ± 0.032	0.498 ± 0.036	0.554 ± 0.035**	0.742 ± 0.042	0.725 ± 0.044	0.809 ± 0.070*	0.799 ± 0.039*

* significantly different from control ($p < 0.05$)

** significantly different from control ($p < 0.01$)

3.12.1.2 Study 2.

Study reference:

Study report, 2015.

Detailed study summary and results:

Test type

Guideline study according to the OECD test guideline 407 and EU Method B.7 (Repeated Dose (28 Days) Toxicity (Oral)), GLP-compliant. Deviation - considered not to affect the scientific integrity of the study. Type of deviation is not stated.

Test substance

- The test material used in the study is equivalent to the substance identified in the CLH dossier: Reaction mass of 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol and benzyl(diethylamino)diphenylphosphonium 4-[1,1,1,3,3,3-hexafluoro-2-(4-hydroxyphenyl)propan-2-yl]phenolate (1:1)
- Degree of purity: > 99%
- Impurities do not affect the classification.
- Batch number: 37060B.

Test animals

- Wistar rats, males and females.
- 5 animals per sex per dose.
- Age at study initiation: approximately six to eight weeks old.
- Weight at study initiation: the males weighed 199 to 217g, the females weighed 157 to 178g.

Administration/exposure

- Route of administration – oral (gavage).
- The test item was administered daily, for up to twenty-eight consecutive days.
- Doses/concentration levels: 100, 300, and 1000 mg/kg bw/day (nominal).
- Rationale for dose level selection: The dose levels were based on the available data from a 7 Day range-finding study. In this study, males given the test item at 500 or 1000 mg/kg bw/day and

females at all dose levels showed body weight impairment that was associated with a reduction in food intake at the start of the dosing period. Subsequent recovery in body weight gain was apparent, however weight gain and food consumption for males receiving the high dose and females at all dose levels were still lower than controls towards the end of the dosing period. In the absence of any clinical signs or macroscopic findings, it is likely that these animals would have attained full recovery over time.

At all dose levels, animals of both sexes receiving the test item also showed a persistent and occasionally dose-related increase in daily water intake in comparison with controls. Taking the overall results into consideration, a high dose level of 1000 mg/kg bw/day was considered to be suitable for use in the present study together with 100 and 300 mg/kg bw/day as the low and intermediate dose levels, respectively. The oral route was selected as the most appropriate route of exposure, based on the physical properties of the test item, and the results of the study are believed to be of value in predicting the likely toxicity of the test item to man.

- Vehicle: Arachis oil
- Control group and treatment: yes, concurrent vehicle
- The stability and homogeneity of the test item formulations were determined. Results show the formulations to be stable for at least eight days when stored at approximately 4 ºC in the dark.

  Formulations were therefore prepared weekly during the treatment period, divided into daily aliquots and stored as above before use. Samples of each test item formulation were taken and analyzed for concentration of XA31.
- Statistical methods: Where considered appropriate, quantitative data was subjected to statistical analysis to detect the significance of intergroup differences from control; statistical significance was achieved at a level of $p<0.05$. Statistical analysis was performed on the following parameters:

  Grip Strength, Motor Activity, Body Weight Change, Hematology, Blood Chemistry, Absolute Organ Weights, Body Weight-Relative Organ Weights.

  Where appropriate, data transformations were performed using the most suitable method. The homogeneity of variance from mean values was analyzed using Bartlett's test. Intergroup variances were assessed using suitable ANOVA, or if required, ANCOVA with appropriate covariates. Any transformed data were analyzed to find the lowest treatment level that showed a significant effect using the Williams Test for parametric data or the Shirley Test for nonparametric data. If no dose response was found but the data shows non-homogeneity of means, the data were analyzed by a stepwise Dunnett's (parametric) or Steel (non-parametric) test to determine significant difference from the control group. For urine volume and specific gravity, pair-wise tests was performed using the Student t-test (parametric) or the Mann-Whitney U test (non-parametric). Urine volume and

specific gravity were statistically analyzed using the R Environment for Statistical Computing. Initially, the distribution of the data was assessed by the Shapiro-Wilk normality test, followed by assessment of the homogeneity of the data using Bartlett's test. Where considered appropriate, parametric analysis of the data was applied incorporating analysis of variance (ANOVA), which if significant, was followed by pairwise comparisons using Dunnett's test. Where parametric analysis of the data was considered to be unsuitable, non-parametric analysis of the data was performed incorporating the Kruskal-Wallis test which if significant was followed by the Mann-Whitney "U" test.

Results and discussion

- There were no clinical observations considered to be related to the toxicity of the test item.
- No mortality observed.
- Body weight and body weight changes: Group mean body weight gains in males from all dose levels were generally lower than controls throughout the study in a dose-related manner resulting in lower overall body weight gains for these animals. An improvement was apparent in males from the 100 mg/kg bw/day dose group towards the end of the treatment period and the effect on body weight development in males from the 300 or 1000 mg/kg bw/day dose groups was considered to be adverse.

  At 300 or 1000 mg/kg bw/day, females showed reduced body weight gains at the start of dosing in a dose-dependent manner. Thereafter, fluctuations were apparent at all dose levels (no dose dependence) and overall weight gains in these females were slightly lower than controls. Taking into consideration the overall results, the effect on body weight development in these females was deemed of no toxicological significance
- Food/water consumption: At 300 or 1000 mg/kg bw/day, food consumption in animals of either sex was marginally reduced during the first week of dosing (dose-related) when compared with controls. Thereafter, an improvement was apparent for most of these animals although food intake for the high dose males remained slightly reduced over the second week. Overall food intake in these animals was slightly lower than controls. Food efficiency in males from all dose groups was generally lower than controls in a dose-related manner with the last week of dosing for the low dose males being an exception. In comparison, treated females only showed a few instances of lower food efficiency following intergroup differences in body weight gains and/or food intake.

  Gravimetric measurement of water consumption revealed episodes of moderate to marked increases in water intake in males treated with 1000 mg/kg bw/day throughout the study and this was considered to be of possible toxicological significance. Females from all dose groups and males from the intermediate dose group also showed instances of slightly increased water consumption in

particular during the first week of dosing; however, these were deemed not to represent an adverse effect of treatment with the test item.

Hematology investigations revealed decreased group mean hemoglobin, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in males and females given 1000 mg/kg bw/day with these females also showing lower hematocrit and mean corpuscular volume. The toxicological significance of these changes was deemed uncertain. Any other changes were deemed unlikely to be of any toxicological significance.
Blood chemistry analysis showed increased plasma concentration of triglyceride in females treated with 300 or 1000 mg/kg bw/day. Some individual values were above the historical data ranges and considering the change was in the opposite direction in the corresponding males (without achieving statistical significance), the toxicological significance of this finding was equivocal. Any other intergroup differences may have been influenced by liver changes and variations in plasma markers and may well be indicative of fluctuations in metabolic processes, but they were considered not to represent an adverse effect of treatment with XA31.

Urine samples from animals of either sex treated with 1000 mg/kg bw/day and females from the 300 mg/kg bw/day dose group were generally slightly more acidic than controls. Additionally, urine samples from one male and two females from the intermediate dose group and two females from the high dose group showed the presence of hemoglobin and in the view of adverse histopathology findings in the kidneys from the intermediate and high dose animals, this finding may be of toxicological significance.

- Behavioral assessment scores across the treated animals of either sex remained similar to the respective controls.There were no treatment-related changes in functional performance at any dose level. Sensory reactivity scores remained unaffected by treatment with the test item.

- Organ weight findings including organ / body weight ratios: At the end of the dosing period, group mean absolute and body weight-related liver weights in animals of both sexes given 1000 mg/kg bw/day, in particular the females, were statisticallysignificantly higher than control. In light of microscopic observations in the liver from these animals, this finding was considered to be of toxicological significance. A dose-related reduction in absolute and body weight-related prostate and seminal vesicles (including coagulating gland) weights was also apparent in males from all dose groups relative to controls. Taking into consideration the histopathology findings from these tissues,

the effect at 300 or 1000 mg/kg bw/day could be of toxicological significance. Any other statistically significant intergroup differences were considered to be of no toxicological importance.

- Treatment-related macroscopic findings of possible toxicological significance included small prostate and seminal vesicles (including the coagulating gland) in males receiving XA31 at dose levels of 300 or 1000 mg/kg bw/day. One male from the high dose group was also observed with small testes and epididymides; the toxicological significance of this finding was deemed equivocal.

- Histopathological findings: Accessory Sex Glands: Minimal to moderate diffuse atrophy of the seminal vesicles, coagulating glands and the prostate gland was present in males given 300 or 1000 mg/kg bw/day. This finding may be a consequence of detrimental effect on dietary intake and body weight development; however, this could not be established with certainty and as such it was considered to be of possible toxicological significance. This observation was not seen in males treated with 100 mg/kg bw/day.
  Kidneys: Minimal to moderate single cell necrosis and minimal incidences of proteinaceouscasts were observed in males given 300 mg/kg bw/day and animals of either sex receiving 1000 mg/kg bw/day. Additionally, minimal to moderate incidences of basophilic tubules were apparent in animals of either sex treated with XA31 at all dose levels. Although basophilic tubules were not seen in the kidneys from control animals, minimal basophilic tubules, without other evidence of degeneration or necrosis, are often seen spontaneously in laboratory rats and it was, therefore, considered likely that the occurrence of this finding in animals from the 100 mg/kg bw/day dose group was not a consequence of administration of the test item.

  Liver: Minimal or mild centrilobular hepatocytic hypertrophy was observed in both sexes receiving 300 or 1000 mg/kg bw/day and females given 100 mg/kg bw/day; minimal or mild basophilia of periportal hepatocytes was also present in most of these animals. At 1000 mg/kg bw/day, minimal bile duct hyperplasia was observed in all animals along with minimal single cell necrosis of the bile duct epithelium in a male and increased hepatocellular mitoses in a female. The centrilobular hypertrophy and periportal basophilia of hepatocytes was considered most likely a consequence of enzyme induction and minor metabolic perturbation and therefore adaptive and non-adverse. When present with bile duct changes, however, it was considered as an adverse change.

  Mammary Gland: Feminine morphology, tubular and ductal profiles with female-like characteristics, were present in segments of the mammary gland from males in all dose group including one control male. According to Lucas et al. (2007), ‘occasionally, ducts and tubules with morphologic

characteristics similar to those observed in female rats are observed in male rats subjacent to normal male lobuloalveolar morphology and should not be diagnosed as feminization', and it is possible that this distribution occurred spontaneously. In males given 1000 mg/kg bw/day, however, some of the female-like ducts were present within the main part of the gland and in view of the findings observed in the accessory sex glands, this finding was considered as possibly test-item related and of possible toxicological significance in these males.

Ovaries: The mild increase in atretic follicles seen in one female treated with 1000 mg/kg bw/day was considered most likely to have arisen by chance.

Testes: Mild spermatid retention, minimal tubular atrophy and minimal atrophy of Leydig cells was observed in one male given 1000 mg/kg bw/day with minimal tubular atrophy on its own also seen in a control male. As with the microscopic findings in the accessory sex glands, this finding could also have resulted from the effects of the food consumption and body weight deficit, and was considered as of equivocal toxicological significance.

Thymus: Minimal lymphocyte depletion observed in two females each from the 300 or 1000 mg/kg bw/day dose groups was considered to be stress-related rather than a direct effect of treatment with XA31.

3.13 Aspiration hazard

Not evaluated in this CLH proposal.

4 ENVIRONMENTAL HAZARDS

Not evaluated in this CLH proposal.