Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Phenol, dodecyl-, branched [1];
Phenol, 2-dodecyl-, branched;
Phenol, 3-dodecyl-, branched;
Phenol, 4-dodecyl-, branched;
Phenol, (tetrapropenyl) derivatives [2]

EC number: 310-154-3 [1]
CAS numbers: 121158-58-5 [1], 74499-35-7 [2]

CLH-O-0000003405-79-03/F

Adopted

5 December 2013

5 December 2013

CLH-O-0000003405-79-03/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemicals name: **Phenol, dodecyl-, branched [1];**
Phenol, 2-dodecyl-, branched;
Phenol, 3-dodecyl-, branched;
Phenol, 4-dodecyl-, branched;
Phenol, (tetrapropenyl) derivatives [2]

EC number: **310-154-3**

CAS numbers: **121158-58-5 [1]; 74499-35-7 [2]**

The proposal was submitted by **Chevron Oronite SAS** and received by the RAC on **5 March 2013.**
In this opinion, all classifications are given firstly in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS) and secondly, according to the notation of 67/548/EEC, the Dangerous Substances Directive (DSD).

PROCESS FOR ADOPTION OF THE OPINION

Chevron Oronite SAS has submitted a CLH dossier containing a proposal together with the justification and background information, documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **5 March 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **19 April 2013**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by the RAC: **Stephen Dungey**

Co-rapporteur, appointed by the RAC: **Bogusław Barański**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation.

The RAC opinion on the proposed harmonised classification and labelling was reached on **5 December 2013** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion on **phenol, dodecyl-, branched; phenol, 2-dodecyl-, branched; phenol, 3-dodecyl-, branched; phenol, 4-dodecyl-, branched; phenol, (tetrapropenyl) derivatives** that should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
Current Annex VI entry	No current Annex VI entry									
Dossier submitters proposal	604-092-00-9	Phenol, dodecyl-, branched [Tetrapropenylphenol (TPP)]	310-154-3	121158-58-5	Repr. 1B	H360F	GHS08 Dgr	H360F	-	Repr. 1B; H360F: C ≥ 1.5 %
RAC opinion		phenol, dodecyl-, branched [1]; phenol, 2-dodecyl-, branched; phenol, 3-dodecyl-, branched; phenol, 4-dodecyl-, branched; phenol, (tetrapropenyl) derivatives [2]	310-154-3 [1]	121158-58-5 [1] 74499-35-7 [2]	Repr. 1B Skin Corr. 1C Aquatic Acute 1 Aquatic Chronic 1	H360F H314 H400 H410	GHS05 GHS08 GHS09 Dgr	H360F H314 H410	-	M=10 M=10
Resulting		phenol, dodecyl-,	310-154-3	121158-58	Repr. 1B	H360F	GHS05	H360F	-	M=10

Annex VI entry if agreed by COM		branched [1]; phenol, 2-dodecyl-, branched; phenol, 3-dodecyl-, branched; phenol, 4-dodecyl-, branched; phenol, (tetrapropenyl) derivatives [2]	[1]	-5 [1] 74499-35-7 [2]	Skin Corr. 1C Eye Dam. 1[1] Aquatic Acute 1 Aquatic Chronic 1	H314 H400 H410	GHS08 GHS09 Dgr	H314 H410		M=10

[1] According to the revised Guidance on the Application of the CLP criteria, a skin corrosive substance is considered to also cause serious eye damage which is indicated in the hazard statement for skin corrosion (H314: Causes severe skin burns and eye damage). Thus, in this case both classifications (Skin Corr. 1 and Eye Dam. 1) are required in the classification column, but the hazard statement H318 'Causes serious eye damage' should not be indicated on the label because of redundancy (CLP, Article 27).

Classification and labelling in accordance with DSD

	Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits
Current Annex VI entry	No current Annex VI entry						
Dossier submitters proposal	604-092-00-9	Phenol, dodecyl-, branched [Tetrapropenylphenol (TPP)]	310-154-3	121158-58-5	Repr. Cat. 2; R60	T R: 60 S: 36/37	Repr. Cat. 2; R60: C > 1.5 %
RAC opinion		phenol, dodecyl-, branched [1]; phenol, 2-dodecyl-, branched; phenol, 3-dodecyl-, branched; phenol, 4-dodecyl-, branched; phenol, (tetrapropenyl) derivatives [2]	310-154-3 [1]	121158-58-5 [1] 74499-35-7 [2]	Repr. Cat. 2; R60 C; R34 N; R50-53	T; N R: 34-50/53-60 S: 45-53-60-61	N; R50-53: C ≥ 2,5 % N; R51-53: 0,25 % ≤ C < 2,5 % R52-53: 0,025 % ≤ C < 0,25 %
Resulting Annex VI entry if agreed by COM		phenol, dodecyl-, branched [1]; phenol, 2-dodecyl-, branched; phenol, 3-dodecyl-, branched; phenol, 4-dodecyl-, branched; phenol, (tetrapropenyl) derivatives [2]	310-154-3 [1]	121158-58-5 [1] 74499-35-7 [2]	Repr. Cat. 2; R60 C; R34 N; R50-53	T; N R: 34-50/53-60 S: 45-53-60-61	N; R50-53: C ≥ 2,5 % N; R51-53: 0,25 % ≤ C < 2,5 % R52-53: 0,025 % ≤ C < 0,25 %

SCIENTIFIC GROUNDS FOR THE OPINION

RAC general comment

Substance identity

The substance is a complex mixture of branched alkyl-substituted phenols, the majority of which are expected to be substituted at the 4- (para) position on the phenol ring. However it is expected that there will also be smaller amounts of 2(ortho)- and 3(meta)- substitution. The alkyl substituent is primarily branched C12 (dodecyl) with an unspecified branching pattern. The harmonised classification will apply to any substance which predominantly contains C12 (branched) alkyl-substituted phenols. For the purposes of this opinion, the substance is called Phenol, dodecyl-, branched (TPP). It is proposed that the Annex VI entry will also specify, under international chemical identification, Phenol, 2-dodecyl-, branched; Phenol, 3-dodecyl-, branched; Phenol, 4-dodecyl-, branched together with the alternative identifier Phenol, (tetrapropenyl) derivatives.

The Dossier submitter (DS) proposed harmonized classification only for reproductive toxicity. Therefore only that proposal is considered in this opinion.

However, RAC adopted an opinion on harmonised classification for additional hazard classes based on a separate CLH dossier for TPP submitted by another DS (SI Group-UK, Ltd). Therefore, the entries in the tables of this opinion for skin irritation, eye irritation and acute and chronic aquatic toxicity are those adopted by RAC, based on the other closely related opinion on TPP.

References used in this opinion are given in full in the Background Document (BD).

HUMAN HEALTH HAZARD ASSESSMENT

Reproductive toxicity

Summary of the Dossier submitter's proposal

According to the DS in this case, exposure to TPP in animal studies resulted in clear evidence of alterations to the onset of puberty, oestrous cyclicity, perturbations to the reproductive system, and impairment of fertility. Although these findings were generally accompanied by reductions in body weight, it was concluded that these effects could not be seen as non-specific secondary effect due to systemic toxicity.

It should be noted that in reproductive studies conducted under various conditions of feed restriction, rats have been repeatedly shown to be resistant to adverse reproductive effects unless there are marked effects upon body weight, typically less than 70% of control body weight. For example, Chapin *et al*. (1993) used feed restriction to control the body weights of adult Sprague-Dawley rats to 90%, 80%, and 70% of control (fed ad lib) rat body weight. This resulted in reduced ovary weight, reduced corpora lutea in the ovary, and prolonged oestrous cycle length only in animals with a body weight reduction of 70% of the control weight. There were no such effects in either group with 80% or 90% body weight compared to that of controls, and no statistically significant effects upon implantations/dam (apparent reduction in implantations at 70% body weight only but not statistically significant).

Given that the reductions in female body weight and body weight gain in the TPP treated animals were similar in magnitude to the reductions where no adverse effects were seen in food deprivation studies (i.e. lower than in the rats where adverse effects were seen), these observed effects on weight cannot account for the observed alterations in reproductive endpoints.

Guidance from ECHA (2008) indicates that mechanistic data may reduce or increase the level of concern about the relevance of a reproductive hazard identified in animal studies to human health. Findings from estrogen-sensitive assays are included in this review. The testing guidelines for these assays were originally developed in response to concerns for human health effects. Thus, results obtained with TPP in these assays should be considered relevant for the interpretation of

reproductive toxicity effects, and included in the weight of evidence when concluding on the classification of this substance.

Two uterotrophic assays (Edwards et al, 2010a and 2010b) were conducted using TPP (as manufactured; purity equivalent to that marketed). The interpretation of the laboratory that conducted both studies was that TPP "demonstrated or mimicked biological activities consistent with agonism of natural estrogens".

Four female pubertal assays were conducted using TPP (as manufactured; purity equivalent to that marketed). From the results, it can be concluded that TPP administered orally to juvenile female rats resulted in estrogenic effects in females as evidenced at 50 and 200 mg/kg bw/day by earlier attainment of vaginal patency (with corresponding lower mean body weight on the day of attainment) and at 200 mg/kg bw/day by first oestrus occurring at a younger age.

TPP is considered a weak androgen receptor (AR) binder based on an *in vitro* rat prostate androgen competitive binding assay and an estrogen receptor (ER) binder based on an *in vitro* rat uterine estrogen receptor competitive binding assay.

Given the large data set available for the evaluation of toxicity to reproduction and given the overall quality of the dataset, classification as Repr. 2 is not considered appropriate. When using a weight of evidence approach, effects can be seen to be dose-responsive, statistically significant, and reproducible across multiple study designs.

The findings include reduced mean live litter size, altered oestrous cyclicity, reduced ovary weights and ovary histology. Increased uterine weight was observed in the uterotrophic assay, as was accelerated sexual maturation in immature animals in the female pubertal assay. Alterations in male reproductive parameters, including effects on multiple accessory organs, sperm production, and transport, were also identified in all studies where this was evaluated, but occurred together with significant effects on general health. Marked systemic toxicity, according to ECHA guidance, include lethality, dramatic reduction in absolute body weight and coma. Therefore, the reductions in parental body weight are insufficient to be the primary cause for the observed changes in reproductive endpoints in females, as several effects occur at body weight reductions of less than 20% relative to controls.

In the opinion of the DS, the weight of evidence from these findings supports, the classification of TPP as Repr. 1B, H360F (adverse effects on sexual function and fertility) according to the CLP Regulation, and Repr. Cat. 2: R60 according to DSD. A Specific Concentration Limit (SCL) of 1.5% was proposed.

The DS did not propose classification for developmental toxicity or adverse effects on or via lactation.

Comments received during public consultation

Comments were received from four MSCAs. Two MSCAs agreed with the proposal of the DS to classify TPP under CLP as Repr. 1B and also with their SCL proposal. Two further MSCAs agreed with the proposed Repr. 1B classification but did not agree with the proposed SCL. They instead proposed to calculate the concentration limit based on the Guidance on the Application of the CLP Criteria. A suggestion for H360 without specification of D or F (Adverse effects on Development or Sexual Function and fertility, respectively) was also made. In addition, the proposed 'no classification' for developmental toxicity (and lactation) was questioned, and the DS was asked to provide more justification for this conclusion.

In their response, the DS provided a new calculation of the ED_{10} values using a 10% effect level by applying linear extrapolation as described in the ECHA guidance (Guidance on the Application of the CLP Criteria (Version 3.0, November 2012, Section 3.7.2.5.3.3) for continuous or parametric data.

The ED_{10} estimations for each study are presented in the table below.

Table 1. Estimation of ED_{10} values using linear extrapolation for ovary weight in rats exposed to TPP in accordance with Knapp (2006) and Edwards et al. (2012)

One-generation study: F0 females ovary and oviduct weight (Knapp, 2006)						
Dose (mg/kg bw/day)	**Number**	**Mean (g)**	**90% of Control (g)**	**Calculation of slope**	**Interpolation**	**ED_{10} (mg/kg bw/day)**
0	30	0.144	0.1296	1250	0.0124	20.5
5	30	0.142				
25	29	0.126				
125	30	0.1				

Two-generation study: F0 females ovary weight (Edwards *et al*., 2012)						
Dose (mg/kg bw/day)	**Number**	**Mean (g)**	**90% of Control (g)**	**Calculation of Slope**	**Interpolation**	**ED_{10} (mg/kg bw/day)**
0	30	0.12	0.108	2041	0.006	27.2
1.5	29	0.121				
15	30	0.114				
75	29	0.0846				

Two-generation study: F1 females ovary weight (Edwards *et al*., 2012)						
Dose (mg/kg bw/day)	**Number**	**Mean (g)**	**90% of Control (g)**	**Calculation of Slope**	**Interpolation**	**ED_{10} (mg/kg bw/day)**
0	30	0.105	0.0945	1583	0.0085	28.5
1.5	30	0.0993				
15	30	0.103				
75	27	0.0651				

The DS noted that the ED_{10} values estimated above from all three studies were still greater than 15 mg/kg bw/day, as derived by them in the original CLH report using the USEPA software BMDS (version 2.3.1; using ovary weight as a critical endpoint in each study). A benchmark response (BMR) of one standard deviation below the control mean value was selected in this previous approach, as according to DS, this adequately, represented the risk of approximately 10% of a population exhibiting a detectable change in a continuous endpoint (Crump, 1995). In the absence of other data, this suggested a toxicologically-relevant threshold for changes in ovary weight.

The DS of the other CLH dossier on TPP, SI Group-UK, Ltd, provided comments in support of their proposed classification as Repr. 2 for fertility. For further details on the argumentation provided, see the opinion on the TPP CLH dossier from the SI Group-UK, Ltd.

Assessment and comparison with the classification criteria

RAC used a weight of evidence approach to classify TPP for reproductive toxicity, taking into account all data provided in the CLH dossiers submitted by both Chevron Oronite SAS and the SI Group-UK, Ltd, respectively.

Adverse effects on sexual function and fertility

Two-generation reproductive toxicity study in rats (OECD TG 416; key study; Klimisch score: 1; Edwards *et al.*, 2012)

In the study of Edwards *et al*. (2012) TPP was administered in the diet of Sprague-Dawley (SD) Crl:CD rats for a minimum of 70 consecutive days at concentrations of 0, 1.5, 15, and 75 mg/kg bw/day in accordance with OECD TG 416. Group sizes were 30/sex for both generations.

F0 males and females were exposed for 129-134 consecutive days, and F1 males and females were exposed for 210 - 227 consecutive days.

Due to reduced fertility in all groups in the second generation, including the control group, the F1 adults were re-bred to produce second litters; the first litters from the F1 adults was referred to as the "F2 litters" while the second litters from these adults was referred to as the "F2a litters".

Following Public Consultation of the SI Group-UK, Ltd dossier, the DS for that dossier, in response to a request from one MSCA provided detailed results of this two-generation study, presented below. RAC noted that the standard deviations were not provided; thus there is no information on variability of the assessed parameters within the experimental groups.

Table 2. Effects on Female reproductive parameters in F0 animals, key findings (Edwards *et al*., 2012)

Parameter F0 females and offspring	**Dose Level (mg/kg bw/day)**			
	0	**1.5**	**15**	**75**
Mean absolute organ weights and microscopic findings (incidences)				
Mean terminal body weight (g)	325	323	321	286** (↓12%)
Mean body weight (g) - initiation of mating	293	290	284	256** (↓12.6%)
Mean body weight gain (g) – initiation of mating	126	123	117	89** (↓29.4%)
Mean ovaries weight (g)	0.1202	0.1210	0.1142	0.0846** (↓30%)
Ovaries – decreased presence of corpora lutea (5 or less)	1/30	0/27	0/30	6/28* (↓18%)
Oestrous cycle length (days) (historical control range: 3.6 – 5.8 days)	4.3	4.3	4.5	5.4**
Persistent oestrus (>3 consecutive days)	1/30	0/30	0/30	0/30
Persistent diestrus (>4 consecutive days)	0/30	0/30	6/30	12/30
Number of implantation sites (measured in F0 only) (historical control range: 12.6 – 17.0)	15.0	14.8	14.7	13.2* (↓12%)
Number born (historical control range: 13.0 – 16.6)	14.0	14.1	14.0	12.5
Live litter size (historical control range: 12.6 - 16.4)	13.8	13.9	13.7	12.2
Pup weight (M/F) – PND 1	7.5/7.0	7.5/7.0	7.3/6.9	7.2/6.8
Pup weight (M/F) – PND 4	10.4/9.8	10.2/9.6	10.0/9.6	9.6/9.0

Parameter F0 females and offspring	Dose Level (mg/kg bw/day)			
	0	1.5	15	75
Mean absolute organ weights and microscopic findings (incidences)				
Pup weight (M/F) – PND 7	16.5/15.7	16.1/15.3	15.1*/14.4**	14.3**/13.5**
Pup weight (M/F) – PND 14	30.1/29.2	30.1/29.1	28.6/27.3	22.9**/21.8**
Pup weight (M/F) – PND 21	50.7/49.3	49.1/46.8	47.0/45.0*	36.4**/34.4**

Statistical significance: *p<0.05; **p<0.01
(historical control range (2000 – 2009) as provided in the study report (Edwards *et al.*, 2012); minimum/maximum values)

Table 3. Effects on Female reproductive parameters – F1 animals, key findings (Edwards *et al*., 2012)

Parameter F1 females and offspring	Dose Level (mg/kg bw/day)			
	0	1.5	15	75
Mean absolute organ weights and microscopic findings (incidences)				
Mean terminal body weight (g)	413	389	383	315** (↓24%)
Mean body weight (g) – initiation of mating	319	313	309	279** (↓12.5%)
Mean body weight gain (g) – initiation of 1st mating	164	167	159	145** (↓11.6%)
Mean ovaries weight (g)	0.1051	0.0993	0.1027	0.0651** (↓38%)
Ovaries – decreased presence of corpora lutea (5 or less)	6/28	2/28	3/30	16/26*
Estrous cycle length (days)	4.3	4.2	4.6	6.5**
Vaginal patency (F1 only) (days)	32.4	32.2	32.4	27.4** (↓15%)
Persistent oestrus (>3 consecutive days)	0/30	0/30	0/30	2/27
Persistent diestrus (>4 consecutive days)	8/30	4/30	9/30	20/27
Number of implantation sites (measured in F0 only)	15.0	14.8	14.7	13.2* (↓12%)
Number of pups born (F2/F2a)	13.4/13.4	13.0/13.1	13.2/13.3	12.6/10.1*
Live litter size(F2/F2a)	13.3/13.4	12.9/12.7	13.0/13.1	12.1/9.5*
F2:				
Pup weight (M/F) – PND 1	7.4/7.0	7.4/6.9	7.1/6.7	6.7*/6.3**
Pup weight (M/F) – PND 4	10.5/9.9	10.8/10.2	10.5/9.6	9.8/9.1
Pup weight (M/F) – PND 7	16.8/15.9	17.4/16.3	16.8/15.3	15.4/14.2*
Pup weight (M/F) – PND 14	33.9/32.5	34.9/33.7	33.7/31.5	29.0**/27.9**
Pup weight (M/F) – PND 21	51.9/49.6	52.6/50.5	52.7/48.9	40.9*/39.4**
F2a				
Pup weight (M/F) – PND 1	7.4/7.0	7.4/7.0	7.1/6.7	7.1/6.8
Pup weight (M/F) – PND 4	10.6/10.0	11.0/10.3	10.1/9.5	10.2/10.1
Pup weight (M/F) – PND 7	16.8/15.8	17.4/16.3	15.6/14.7	15.3/15.2
Pup weight (M/F) – PND 14	33.9/32.5	34.9/33.2	31.8/30.7	28.4**/28.4*
Pup weight (M/F) – PND 21	53.1/50.0	54.3/51.3	51.2/48.3	42.8**/42.1**

Statistical significance: *p<0.05; **p<0.01

Table 4. Effects on Male reproductive parameters – F0 animals, key findings (Edwards *et al*., 2012)

F0 Males (F1 offspring)	Dose Level (mg/kg bw/day)			
	0	1.5	15	75
Mean organ absolute weights and microscopic findings (incidences)				
Mean terminal body weight (g)	616	623	611	502** (↓18.5%)
Mean testes weight (g) left	1.79	1.69	1.75	1.62* (↓5%)
Mean testes weight (g) right	1.78	1.74	1.70	1.66
Mean epididymis weight (g) left	0.75	0.72	0.76	0.63** (↓16%)
Mean epididymis weight (g) right	0.79	0.76	0.79	0.68** (↓13.9%)
Epididymis sperm concentration (x106/g) left	365.2	333.6	357.3	288.5* (↓26%)
Mean cauda epididymis weight (g) left	0.3666	0.3339	0.3755	0.2747** (↓25%)
Mean cauda epididymis weight (g) right	0.3671	0.3529	0.3686	0.2838** (↓23%)
Mean cauda epididymis weight relative to body weight (g/100g) left	0.060	0.054	0.062	0.055
Mean cauda epididymis weight relative to body weight (g/100g) right	0.060	0.057	0.061	0.057
Mean cauda epididymis weight relative to brain weight (g/100g)left	16.892	15.530	17.450	12.818** (↓24%)
Mean cauda epididymis weight relative to brain weight (g/100g) Right	16.885	16.483	17.137	13.235** (↓22%)
Mean prostate weight (g)	1.13	1.09	1.09	0.88** (↓22%)
Mean prostate weight relative to brain weight (g/100g)	51.959	50.983	50.633	41.039** (↓21%)
Mean seminal vesicle weight (g)	2.34	2.22	2.31	1.74** (↓26%)
Mean seminal vesicle weight relative to body weight (g/100g)	0.404	0.359	0.379	0.346 (↓14%)

Statistical significance: *p<0.05; **p<0.01

Table 5. Effects on Male Reproductive Parameters – F1 animals, key findings (Edwards *et al*., 2012)

Parameter F1 Males	Dose Level (mg/kg bw/day)			
	0	1.5	15	75
Mean organ absolute weights and microscopic findings (incidence)				
Mean terminal body weight (g)	791	814	754	566** (↓28.4%)
Mean body weight (g) - initiation of mating	543	545	536	449** (↓17.3%)
Mean testes weight (g) left	1.87	1.94	1.94	1.74
Mean testes weight (g) right	1.93	1.96	1.88	1.72** (↓11%)
Mean epididymis weight (g) left	0.67	0.73	0.75* (↑12%)	0.65
Mean epididymis weight (g) right	0.76	0.80	0.77	0.68** (↓10.5%)
Epididymis sperm concentration (x106/g) left	310.1	339.4	350.2	320.5

Parameter F1 Males	Dose Level (mg/kg bw/day)			
	0	1.5	15	75
Mean organ absolute weights and microscopic findings (incidence)				
Mean cauda epididymis weight (g) left	0.3028	0.3362	0.3391* (↑12%)	0.2740
Mean cauda epididymis weight (g) right	0.3349	0.3588	0.3372	0.2879** (↓14%)
Mean cauda epididymis weight relative to body weight (g/100g) left	0.039	0.042	0.046**	0.049** (↑25%)
Mean cauda epididymis weight relative to body weight (g/100g) right	0.043	0.045	0.045	0.052** (↑21%)
Mean cauda epididymis weight relative to brain weight (g/100g) left	13.751	15.663* (↑14%)	15.825** (↑15%)	13.116
Mean cauda epididymis weight relative to brain weight (g/100g) right	15.253	16.714	15.720	13.815
Mean prostate weight (g)	1.06	1.07	1.06	0.92* (↓13%)
Mean prostate weight relative to brain weight (g/100g)	48.133	49.916	49.262	44.003
Mean seminal vesicle weight (g)	2.19	2.26	2.2	1.81** (↓17)
Mean seminal weight relative to body weight (g/100g) right	0.28	0.284	0.296	0.32** (↑14%)

Statistical significance: *p<0.05; **p<0.01

The values on assessment of mating, fertility indexes and gestation length in the two-generation study (Edwards *et al*., 2012) were not provided in the CLH dossier on TPP submitted by Chevron Oronite SAS. In the CLH dossier submitted by the SI Group-UK, Ltd, it is stated that reproductive indices in the F0 generation were unaffected by treatment at dose levels up to 75 mg/kg bw/day. Fertility indices in the F0 generation were slightly lower at 75 mg/kg bw/day but values did not attain statistical significance and were within the laboratory's historical control range. Gestation length was unaffected by the treatment. In the F1 generation the reproduction indices for the first mating (F1 generation) were lower than controls at 1.5 and 15 mg/kg bw/day, but because values at 75 mg/kg bw/day were comparable to controls and a clear dose-response relationship could not be demonstrated, a second mating was performed (the same animals were paired) to clarify the significance of these findings. Following the second mating of the F1 generation, reproduction indices in animals at 1.5 and 15 mg/kg bw/day were slightly higher compared to controls. Fertility and copulation indices at 75 mg/kg bw/day were not significantly lower than in controls but values in all groups were low as a consequence of the age of animals at the second mating, and hence data for this second mating cannot be considered as robust. Gestation length was unaffected by treatment in both the first and second mating.

It is concluded that in this two-generation study mating and fertility indexes and gestation length were unaffected by treatment in rats at doses 1.5, 15 and 75 mg/kg bw/day, although marked parental toxicity was noted at 75 mg/kg bw, as can be inferred from 12.6% and 12.5% reduction of body weight of F0 and F1 females at the initiation of mating, respectively and from 18.5% and 28.4% reduction of body weight of F0 and F1 males at a dose of 75 mg/kg bw at termination, respectively.

The number of pups born and live litter sizes were statistically significantly reduced at 75 mg/kg bw/day for the F2a litters compared to controls (13.4 versus 10.1 and 13.4 versus 9.5, respectively). These values were also lower, but not statistically significant, in the F1 and F2 litters. In F0 females of the 75 mg/kg bw/day group, there was a statistically significant reduction in the mean number of implantation sites (13.2 vs. 15 in controls). F1 dams were not evaluated for implantation sites due to multiple gestations.

It is noted that among the three generations of litters observed in the two-generation study, in one generation (F2a) there was a reduction of litter size at 75 mg/kg bw/day, and there was a decrease in the mean number of implantation sites in F0 females at 75 mg/kg bw/day. These values were well within the historical control range (12.6-17.00 for implantation sites and 12.6 –

16.4 for live litter size). Thus the effect of TPP on the litter size in 1 out of 3 generations of litters observed only at 75 mg/kg bw/day, which also caused clear maternal toxicity, does not provide a strong presumption that the substance interferes with fertility.

One-generation reproductive toxicity study in rats (OECD TG 415; supporting study, Klimisch score: 1, Knapp, 2006)

In the study of Knapp, 3 groups of SD Crl:CD rats (30 males and 30 females per group) were administered the test substance daily by oral gavage for 73 consecutive days prior to mating. The one-generation study was designed to meet or exceed the testing requirements of the OECD TG 415. Both sexes of the parental generation were treated with doses of 0 (corn oil vehicle), 5, 25 or 125 mg/kg bw/day by oral gavage (5ml/kg dosage volume). Males were dosed daily until euthanasia. Female rats were dosed through mating, gestation, and lactation until euthanasia. Oestrous cyclicity was evaluated prior to mating while oestrous cycle stage and semen quality were evaluated at necropsy. Due to marked effects on reproduction, selected offspring were retained post-weaning without dosing for evaluation of sexual maturation landmarks; vaginal opening or preputial separation.

Table 6. One-generation study - findings in parental animals according to Knapp (2006)

Observation	**Sex**	**Dose level** (mg/kg bw/day)			
		0	**5**	**25**	**125**
Signs of toxicity	M	-	-	✔	✔
	F	-	-	✔	✔
Pre-mating bodyweight (g)	M	530.3	531.1	505.9	421.2** (79.4%)
	F	287.1	281.4	284.2	259.3** (90.3%)
Terminal bodyweight (g)	M	653.4	638.3	569.2**	467.5**
Pre-mating weight gain (g)	M	355.3	355.2	330.5*	247.0**
	F	130.8	125.9	127.9	103.0**
Overall weight gain (g)	M	460.4	462.4	393.3**	293.3**
Evidence of mating (#)	M	30	28	28	28
	F	30	28	28	28
Pre-coital interval (days)	M/F	3.6	2.6	2.8	2.7
Mating index (%)	M	100	93.3	93.3	93.3
	F	100	93.3	93.3	93.3
Fertility index (%)	M	93.3	90.0	83.3	13.3**
	F	93.3	90.0	83.3	13.3**
Copulation index (%)	M	93.3	85.7	89.3	14.3**
	F	93.3	85.7	89.3	14.3**
Oestrus cycle (days)	F	4.4	4.6	4.9	5.2
Persistent oestrus (#)	F	0	0	0	6
Persistent diestrus (#)	F	2	2	4	16
Gestation length (days)	F	21.9	21.7	21.7	22.3

Statistical significance: *p<0.05; **p<0.01

Shading illustrates the doses of TPP that resulted in evidence of both systemic toxicity and effects on reproduction parameters.

There were no effects on mating behaviour at any dose level. Fertility and mean litter size were unaffected at 5 and 25 mg/kg bw/day. Male and female rats dosed by gavage with 125 mg/kg bw/day showed a marked reduction in fertility; only 4/30 pairs of rats with evidence of copulation resulted in a pregnancy compared to 28/30 of control pairs. Mean litter size was reduced to 1.7 pups per litter at 125 mg/kg bw/day compared to 13 pups per litter in controls.

The body weight was reduced by 9.7% at initiation of mating and 18.5% at termination in females exposed at 125 mg/kg bw/day. The effect upon body weight (maximum decrease to 82% of body weight of control animals at termination) is considered insufficient to be the cause of the reduction in ovary weight. Studies in rats evaluating the effects of feed restriction have demonstrated that female body weight must be reduced to approximately 70% of control before ovary weight will decrease (Chapin, 1993; Seki *et al.,* 1997).

The adverse effect on fertility in the adult rats was accompanied by adverse microscopic changes in both male and female reproductive organs, adverse effects on female cyclicity, and a reduction

in epididymal sperm concentration (effects described below). The reduction in fertility and effects of reproductive organs occurred at doses that also induced other toxic effects, including reduced body weight gain and food consumption and changes in the adrenals, kidneys and liver. However, this toxicity to non-reproductive organs was insufficient to deem the reproductive findings as secondary non-specific effects.

It is concluded that in this one-generation study the mating index was unaffected at all doses, but the fertility index was reduced to 13.3% (93.3% in control group) at 125 mg/kg bw/day. Moderate maternal toxicity was noted at 125 mg/kg bw, as can be inferred based on 9.7% reduction of body weight of parental females at the initiation of mating. Markedly reduced fertility at 125 mg/kg indicates that TPP at a dose moderately toxic to rats can affect fertility.

90-day repeated dose toxicity study in rats (Haas, 2007)
Study design: SD rats (10 animals/sex/dose) were exposed to 0, 50, 100, 150 and 200 mg/kg bw/day TPP in the diet for 91-92 consecutive days. This study was performed to provide guidance on dose-selection for the two-generation study in rats (Edwards *et al.*, 2012), and therefore not all parameters included in the OECD TG 408 were examined. No analysis of semen or oestrous cyclicity was done.

At the highest dose, 200 mg/kg bw/day, there was a disproportionate high number of female rats in oestrus (7/10 vs. 2/10 in the concurrent control group) at necropsy. This was not statistically significant, but it is a biologically relevant observation. Ovary weights were reduced in a dose-dependent manner at 100, 150, and 200 mg/kg bw/day; microscopically, fewer corpora lutea were present at 150 and 200 mg/kg bw/day (in 4/10 and 7/10 females, respectively, vs. 1/10 control). Uterine weights were reduced (not statistically significant) at 150 and 200 mg/kg bw/day, without associated macroscopic or microscopic findings.

Other findings in female rats included reduced body weight and body weight gain at all dosages (approx. 90% to 81% of control body weight at termination), reduced food consumption at 100, 150, and 200 mg/kg bw/day (approx. 90% to 85% of control), liver vacuolization at 150 and 200 mg/kg bw/day, reductions in white blood cells and lymphocytes at 200 mg/kg bw/day, and dose-responsive reductions in serum cholesterol at 100 - 200 mg/kg bw/day.

28-day repeated dose toxicity study in rats (Harriman, 2004)
Study design: SD Crl:CD IGS BR rats were exposed by oral gavage to 0, 5, 20, 60, 180 and 300 mg/kg bw/day, 7 days a week for 4 weeks, according to OECD TG 407. (10 animals/sex in 0 and 300 mg/kg groups; 5/sex/group terminated at 28 days, 5/sex/group terminated after 14-day recovery period; 5/sex/group in other dose groups), study designed to provide guidance for dose selection for the subsequent one-generation oral (gavage) reproduction study.

There was overt toxicity at the top two doses, as evidenced by decreased cumulative mean body weight gains that resulted in mean lower body weights (statistically significant in males only, 13% and 10% reductions at 180 and 300 mg/kg bw/day, respectively). Changes observed only in females included decreased haematocrit and haemoglobin, decreased serum cholesterol, and increased serum triglycerides. These changes were observed at 180 and 300 mg/kg bw/day in a dose-responsive pattern. Mean haemoglobin values (g/dl) were statistically significantly lower than control values (by 9-12%) in females treated with 180 and 300 mg/kg bw/day.

There was no statistically significant increase in adrenal gland weight in females at any dosage. Liver weights increased with dose, becoming statistically significant in males and females at 300 mg/kg bw/day, compared to controls. The increase in liver weights coincided with an increased incidence of animals with centrilobular hepatocellular hypertrophy (males: 0/5, 0/5, 2/5, 2/5 and 5/5, females: 0/5, NE, 0/5, 4/5 and 5/5 at 0, 20, 60, 180 and 300 mg/kg bw/day, respectively) and periportal hepatocellular vacuolization (males: 0/5, 0/5, 0/5, 0/5 and 3/5, females: 0/5, NE, 0/5, 0/5 and 1/5 at 0, 20, 60, 180 and 300 mg/kg bw/day, respectively).

The incidence in the number of male rats with follicular cell hypertrophy in the thyroid increased with dose (0/5, 1/5, 1/5, 2/5, 3/5 and 3/5 at 0, 5, 20, 60, 180 and 300 mg/kg bw/day, respectively) but these changes were not observed in females. Follicular cell hypertrophy tends to be a transient finding in rats and has limited relevance to human hazard identification.

Mean ovary weight was reduced at 180 and 300 mg/kg bw/day in a dose-responsive pattern. The change in ovarian weight was accompanied by reduced corpora lutea observed microscopically.

90-day study in rats with oral exposure in diet (Vogin, 1970a):
Study design: FDRL rats (20/sex/group), 90-day treatment via diet containing 0, 0.05, 0.2 and 0.4% of TPP (equivalent to 0, 25, 100 and 200 mg/kg bw/day) 7 days/week. Test material: Phenol, dodecyl (CAS 27193-86-8).
No deaths occurred and no clinical observations of toxicity were observed during the study period. Weight gain and food utilisation efficiency was reduced at 200 mg/kg bw/day in males (81.6% of control males' body weight) and females (89% of control females' body weight). Mean absolute and relative testes weights were reduced in males at 200 mg/kg bw/day with testicular hypospermia observed in 6 out of 20 animals. Additionally, liver weights were increased among either sex at 200 mg/kg bw/day. No additional histopathological effects were noted in this study. A NOAEL of 100 mg/kg bw/day was assigned for general toxicity and effects on the male reproductive tract.
The results of this study indicate that the effect of the test material on the male reproductive tract at the highest dose level of 200 mg/kg bw/day was associated with reduced weight gain.

90-day study in dogs with oral exposure in diet (Vogin, 1970b):
Study design: Young Beagle dogs (3/sex) were administered TPP at dietary concentrations of 0, 0.05, 0.2 and 0.4%, equivalent to calculated mean intakes of approximately 0, 18, 71 and 143 mg/kg bw/day respectively; test material: Phenol, dodecyl (CAS 27193-86-8); 13 week treatment duration; treated feed was available 1 h/day, 6 days/week.

No deaths occurred and no signs of toxicity were observed during the study period. Bodyweight gains were unaffected by treatment. No treatment-related effects were apparent on either organ weights or in histopathology assessment. Although the study is older than the preceding 90-day study in the rat (Haas, 2007), relevant investigations (weights and histopathology of the testes and associated tissues) were performed and the study is considered to be adequate for the assessment of general toxicity and effects on the male reproductive tract. It is noted that the 90-day rat study (of similar design) performed at this laboratory and at a similar time detected effects on the male reproductive tract comparable to those observed in more recent studies.

A NOAEL of > 143 mg/kg bw/day was assigned for general systemic toxicity and effects on the male dog reproductive tract. Although this repeated dose dietary study in dogs suggests that the effects of TPP observed in rat studies could plausibly be due to species-specific sensitivity and calls into question the relevance of findings in rat studies to humans, it should be noted that only three dogs were used in each group.

Summary of effects on female fertility

In the two-generation study (Edwards *et al*., 2012), alterations to female reproduction included lengthened oestrous cycles at 75 mg/kg bw/day, as well as an increase in the number of female rats in persistent diestrus. These changes were observed in both generations of adult female rats. Also the ovary weight and the number of corpora lutea were reduced at 75 mg/kg in both generations. The reduction in body weight in the F0 and F1 females (88% and 76% of the control values, respectively) was insufficient to account for the microscopic findings or reduced ovary weights in the F0 and F1 females (71% and 62% of the control values, respectively). Vaginal patency occurred earlier in the F1 offspring at 75 mg/kg bw/day (27.4 days versus 32.4 days in controls).

In the one-generation study by Knapp (2006), mean absolute ovarian weight was significantly reduced in females at 25 and 125 mg/kg bw/day (87% and 70%, respectively, of control values). Microscopic evaluation of ovaries revealed an increase in ovarian cysts (in 15/30 animals vs. 4/30 in controls) and decreased corpora lutea (in 18/30 animals vs. 4/30 in controls) at 125 mg/kg bw/day. Uterine weight was unaffected, although this measure may not have been valid due to differences between exposure groups in proportions of rats that had produced litters. Microscopically, an increase in endometrial gland cysts (8/30 animals vs. 1/30 in controls) was detected at 125 mg/kg bw/day. At 125 mg/kg bw/day, a disproportionate number of females, many of which had mated but did not show evidence of pregnancy (implantation sites at necropsy), were in oestrus at termination (16/30 vs. 3/30 in controls). This finding mirrored

changes to oestrous cyclicity detected during weeks 7-10 of exposure. At the mid and high dose, oestrous cycle length increased (4.9 and 5.2 days, respectively, vs. 4.4 days in controls). In the high dose group, 6/30 females and 16/30 females displayed persistent oestrus or diestrus, respectively, and 6/30 females were essentially acyclic (vs. 0/30, 2/30, and 0/30 for each endpoint, respectively, in controls).

Other findings included red material in the facial area, reductions in body weight (at 125 mg/kg bw/day, females had 90% of control body weight at initiation of mating), reduced food consumption that mirrored the body weight gain reductions, and reduced food efficiency during the first weeks of exposure. Non-reproductive organ effects included decreased absolute liver weight (the relative liver weight was increased) at 25 and 125 mg/kg bw/day without microscopic changes and reduced absolute kidney weight (the relative kidney weight was increased) at 125 mg/kg bw/day with evidence of renal mineralization (7/30 vs. 1/30 in control).

The analysis of data provided in both CLH reports submitted (by Chevron Oronite SAS and SI Group-UK, Ltd, respectively) and during PC indicates that a considerable food restriction and reduction in body weight of female rats may have influenced their sexual function. Feed restrictions in SD rats leading to a 70% reduction in body weight as compared to controls had no effect on fertility. However, a decreased ovary weight and decreased number of corpora lutea as well as a transient prolongation of the oestrous cycle time were seen in female rats that weighed 70% of controls but not in rats that weighed 80 or 90% of control females (Chapin *et al*., 1993) Decreased body weight induced by feed restriction in female rats may induce a decrease in ovary weight and number of corpora lutea (Terry *et al*., 2005; Seki *et al*., 1997; Chapin *et al*., 1993), an increase in oestrus cycle length (Terry *et al*., 2005; Seki *et al*., 1997) and result in generally decreased reproductive performance (Guzman, 2006; Zambrano *et al*., 2005; Aiguo *et al*., 2002). For example, Terry *et al.* (2005) reported on compromised fertility due to reduction in the number of corpora lutea associated with only a 16% decrease in body weight which is not far from those reported in one- and two-generation and repeated dose toxicity studies for TPP (9.7 – 12.5%). Nevertheless the reductions in ovary weight and in the number of corpora lutea in females treated with TPP cannot be explained only by reduced feed consumption and reduced body weight compared with control females; and thus they are concluded to be treatment related.

Summary of effects on male fertility

Both in the feed restriction studies and in the TPP reproduction and repeated-exposure studies, there was a decrease in accessory reproductive organ weights which was proportionate to the decrease in body weight. For this reason, RAC did not base the classification for fertility on the effects seen in males.

In the two-generation study (Edwards *et al*., 2012), test substance-related organ weight changes at 75 mg/kg bw/day consisted of lower weights of the left and right epididymides (14-16% of control values) and cauda epididymides (by 23-25%), prostate (21%), and seminal vesicles (26 -17 %)/coagulating glands in F0 and F1 males, and lower left and right testes weights in F1 males. Mean epididymal sperm concentration was also lower in the 75 mg/kg bw/day dose group. These changes occurred together with reduced body weight. The reduction in terminal body weight of male rats was 18.5% in the F0 and 28.4% in the F1, relative to the concurrent control, which is of similar magnitude to the reductions observed in the male accessory sex organ weights relative to the control values (10.5% to 25%, respectively). Consequently there were few statistically significant differences when accessory reproductive organ weights were evaluated relative to control values.

No histopathological findings were identified as treatment-related in the reproductive organs. The sole histopathological finding in males that was attributed to TPP was renal mineralization in F0 males at 75 mg/kg bw/day and in F1 males at 15 and 75 mg/kg bw/day, a finding frequently seen in female rats but less commonly observed in males (the effect was not attributed to treatment in females in this study).

In the one-generation study (Knapp, 2006) at 25 mg/kg bw/day, there was a significant decrease in the mean cauda epididymides absolute weight compared to controls, which was also significantly reduced relative to brain weight. Histopathological findings at this dose level included a significant increase in the number of animals with decreased secretions in the coagulating and prostate glands compared to controls.

At the highest dose of 125 mg/kg bw/day, the mean testes and epididymides absolute weights were significantly decreased compared to controls. More informatively, significant decreases in testes and epididymides weights relative to brain weight were also observed at this dose level. Additionally, mean epididymal sperm concentration was significantly reduced from 365.2 x 10^6/g in controls to 303.2 x 10^6/g in the highest dose group. Also, there was a significant increase in the number of animals with microscopic findings of decreased secretions in the seminal vesicle glands compared to controls. As noted below, this may, in part, be associated with body weight effects. Male accessory reproductive organ weights, particularly the seminal vesicles and prostate, are sensitive to body weight changes. This sensitivity may be due to the proportion of glandular luminal content (fluid) relative to organ mass (Chapin *et al.,* 1993; Rehm *et al.,* 2008). Consequently, effects upon male accessory organs are interpreted with caution.

In the 90-day repeated dose toxicity study in rats (Haas *et al.*, 2007) findings at necropsy included small coagulating glands, prostate and seminal vesicles in the 150 and 200 mg/kg bw/day dose groups and small epididymides and testes in the 200 mg/kg bw/day dose groups. Reductions in absolute testes weight (by 36%) and in relative testes weight along with other changes in the testes included atrophy and hypospermia in the 200 mg/kg bw/day dose group. Reduced prostate and seminal vesicle weights (relative and absolute) were noted at 100, 150 and 200 mg/kg bw/day while testes weights were increased at 100 and 150 mg/kg bw/day as compared to controls. These results are interpreted with caution since, as said above, male accessory reproductive organ weights are sensitive to changes in body weight. Microscopic findings included hypospermia in the testes in 2/20 animals at the 100 mg/kg bw/day dose, and hypertrophy of coagulating gland and atrophy of the prostate at 200 mg/kg bw/day. Decreased seminal vesicle secretions were seen in the 150 and 200 mg/kg bw/day dose groups as well. Renal mineralization, normally more commonly observed in females, was observed only in male kidneys at all doses investigated.

In the 28-day repeated dose toxicity study in rats (Harriman, 2004), mean testes weights were statistically significantly reduced by 42% in males at 300 mg/kg bw/day accompanied by germ cell depletion and interstitial cell atrophy. Mean testes weights were reduced by 15% in males at 180 mg/kg bw/day, and although the reduction was not statistically significant, it was accompanied by interstitial cell atrophy (0/5, 0/5, 0/5, 5/5, and 4/5) and depletion of mature germ cells (0/5, 0/5, 0/5, 1/5, 4/5). There was also a low (1/5) incidence of animals with microscopic degeneration of the seminiferous tubules in the testes at all dose levels, although this effect showed no dose-response over the 5 to 300 mg/kg bw/day dose range.

In males treated with 180 and 300 mg/kg bw/day, statistically significant reductions were observed in mean epididymides weights (by 28% and 58%), seminal vesicle weights (by 67% and 79%), and prostate weights (by 56% and 78%). These reductions were accompanied by an increased incidence in microscopic observations of decreased secretion in the seminal vesicles, coagulating gland, and prostate. There were increased incidences in animals with hypospermia and cellular luminal debris in the epididymides at 300 mg/kg bw/day. Relative weights of male reproductive accessory organs, as a percentage change from control, were substantially more affected than terminal body weights.

In the Vogin (1970a) 90-day study in rats there was an effect of the test material on the male reproductive tract at the highest dose level of 200 mg/kg bw/day, but this was considered to be associated with the reduced body weight gain.

Effects of body weight reduction on reproductive organ weights – background information

Several publications which have examined the relationship between body weight changes and male reproductive organ weight changes in the rat (Scharer, 1977; Chapin *et al*., 1993; Levin *et al*., 1993; Keenan *et al*., 1994; Seki *et al*., 1997; Odum *et al*., 2001; Marty *et al*., 2003; Carney *et al*., 2004; Terry *et al*., 2005; Laws *et al*., 2007) have been summarized in OECD draft guidance document 151 (http://www.oecd.org/env/ehs/testing/GD%20151_Oct%202012_clean2.pdf). These studies showed that reductions in the weights of testes and epididymides were usually smaller than reductions in body weight. A 15% body weight reduction was correlated with a testes and epididymides weights reduction of 2-12%; a 40% body weight reduction resulted in testes and epididymides weights being reduced by 24%. Prostate and seminal vesicle weight varied

more with body weight. At 10% body weight reduction, prostate and seminal vesicle weights were reduced by 0-20% and at 40% body weight reduction, prostate and seminal vesicle weights were reduced by 20-45%.

In the opinion of RAC, the comparison of the effects seen in studies with TPP, and the effects seen in food restriction studies, on the reduction of testes weight and accessory sex organ weights strongly suggest that most of the effects observed in TPP exposed male rats can be attributed to the reduction of body weight and food consumption. Thus the available data do not provide strong evidence of the reproductive toxicity of TPP in male rats.

Mechanistic Studies Related to Reproductive Toxicity

Uterotrophic bioassay (OECD TG 440; supporting study; Klimisch score: 1, Edwards *et al*. (2010a)

Study design: Six ovariectomized female Crl:CD(SD) rats were exposed to 0, 75, 125, 250 or 500 mg/kg bw/day of TPP (tetrapropenyl phenol) once daily for 3 consecutive days by oral gavage. The positive control group received 0.2 mg/kg bw/day of 17α-ethynylestradiol. Females were approximately 42 days of age at the time of ovariectomy and approximately 60 days of age at the beginning of test substance administration.

Dose-dependent increases in wet (181% - 739%) and blotted (183% - 275%) mean uterine weights at all exposure levels were reported when compared to the vehicle control group. The positive control substance (17α-ethynylestradiol) also elicited the expected increase in uterine weights (wet and blotted), but the percentage of the increase was not provided for that group.

Uterotrophic bioassay in rats, (OECD TG 440; supporting study; Klimisch score: 1, Edwards *et al*., 2010b)

Study design: Four groups of six ovariectomized female Crl:CD(SD) rats were exposed to 0, 75, 125, 250 or 500 mg/kg bw/day (actual ingested dose) of purified TPP once daily for three consecutive days by oral gavage. The positive control group was composed of six ovariectomized females and received 0.2 mg/kg bw/day of 17α-ethynylestradiol by oral gavage. The females were approximately 45 days of age at the time of ovariectomy (performed by the supplier) and approximately 60-64 days of age at the beginning of test substance administration.

Dose-dependent increases in wet (177% - 508% of control value) and blotted (184% - 251 % of control value) mean uterine weights were seen at all exposure levels compared to the vehicle control group. The positive control substance (17α-ethynylestradiol) elicited the expected increases in uterine weights (wet and blotted), but the percentage of increase was not provided for that group.

However, the percentages of increases in uterine weights were the same in all dose groups and the actual weighs of wet and blotted uterine were not reported.

Summary of effects in the uterotrophic bioassays

RAC notes that the results indicate some estrogenic activity of TPP, however the potency of this action is very difficult to assess, since the magnitude of the response in the positive control was not provided. Roughly it may be estimated, assuming the same magnitude of response in a group of 75 mg TPP/kg/day and in the group of 0.2 mg/kg bw/day of 17α-ethynylestradiol, that the estrogenic activity of TPP relative to 17α-ethynylestradiol is 75/0.2, i.e. the estrogenic activity of TPP is about 375 times lower than that of 17α-ethynylestradiol. The lowest dose of TPP exhibiting estrogenic activity was considered as toxic to female rats based on reduced body weight in comparison to controls.

Female Pubertal Assay in rats (supporting study, Klimisch score: 1; Knapp, 2009a, GLP compliant)

Study design: Female SD rats were exposed by oral gavage to 10, 50, 200 or 800 mg/kg bw/day of TPP (purified, concentrated C12 homolog >85%) once daily for 20 consecutive days during PND 22-41.

Estrogenic effects were induced at 50 and 200 mg/kg bw/day as evidenced by earlier attainment of vaginal patency (lower mean body weight on the day of vaginal patency attainment) and by younger age at the first occurrence of oestrus at 200 mg/kg bw/day. There was systemic toxicity at 200 and 800 mg/kg as shown by reduced body weight in females at 200 mg/kg and lethality at 800 mg/kg.

At 200 mg/kg bw/day 12/15 females exhibited persistent oestrus (≥3 consecutive days of oestrus). No treatment-related effects on mean serum E2, LH, T4 or TSH levels were observed at any dose level. At 200 mg/kg bw/day mean absolute and relative wet and blotted uterus weights (and thus, luminal fluid weight) and thymus gland weights were lower than in the controls.

Lower mean absolute ovary/oviduct weights were observed in the 50 and 200 mg/kg bw/day groups. In the 200 mg/kg bw/day group, morphologic changes (absent corpora lutea, oocyte degeneration, granulosa cell necrosis) in ovaries were present.

Female Pubertal Assay in rats (supporting study, Klimisch score: 1; Knapp, 2009b, GLP compliant)

Study design: Crl:CD(SD) immature female rats were exposed to 0, 10, 50, 200 or 800 mg/kg bw/day of distilled TPP (concentrated C15 homolog >85%) by oral gavage once daily for 20 consecutive days during PND 22-41. Estrogenic effects were seen in females at 50 and 200 mg/kg bw/day as evidenced by earlier attainment of vaginal patency (lower mean body weight on the day of vaginal patency attainment) and by younger age at the first occurrence of oestrus. At 200 mg/kg bw/day mean absolute and/or relative (to final body weight) wet and blotted uterus weights (and thus, luminal fluid weight), ovary/oviduct, spleen weights and thymus gland weights were lower than in the controls.

There was systemic toxicity at 200 and 800mg/kg as shown by reduced body weight in females at 200mg/kg and lethality at 800 mg/kg.

Although lower mean absolute ovary/oviduct weights and wet and/or blotted uterus weights did not occur in a dose-related manner in the 10 and 50 mg/kg bw/day groups, the reductions in these weights were considered treatment-related. No test substance-related effects on mean serum E2, LH, T4 or TSH levels were observed at 10, 50 or 200 mg/kg bw/day.

Microscopic correlates in the ovary included absence or reduction in the number of corpora lutea, degeneration of oocytes and necrosis of granulosa cells in ovarian follicles at 200 mg/kg bw/day.

Female Pubertal Assay in rats (supporting study, Klimisch score: 1; Knapp, 2007a)

Study design: Crl:CD (SD) immature female rats were exposed to 5, 20, or 60 mg/kg bw/day of calcium salt of TPP once daily for 20 consecutive days (PND 22-41) by oral gavage.

Acceleration of vaginal patency was observed at 60 mg/kg bw/day (attained at 29.1 days vs. 33.2 days in the control group). TPP administration did not affect body weight, but since the vaginal patency was attained at a younger age, there was also a significant reduction in body weight at attainment (89 g vs. 111 g in the control group). There were no changes in organ weights (liver, kidneys, adrenal glands, uterus, ovaries, pituitary or thyroid).

Microscopically, reductions in corpora lutea were noted at 20 and 60 mg/kg bw/day (in 3/15 and 4/15 animals, respectively, vs. 1/15 in control) and uterine hypoplasia occurred at 60 mg/kg bw/day (7/15 vs. 2/15 in control).

Other findings were thyroid gland follicular cell hypertrophy at 60 mg/kg bw/day (10/15 vs. 1/15 control), which was not associated with changes in serum T4 or TSH concentrations.

The study authors concluded that TPP "exhibited slight estrogenic effects" at the highest dose tested.

Female Pubertal Assay in rats (supporting study; Klimisch score: 1, Knapp, 2007b)

Study design: Crl:CD (SD) IGS BR immature female rats were exposed by oral gavage to 0, 60, 250 or 1000 mg/kg bw/day of calcium salt of TPP once daily for 20 consecutive days (PND 22-41).

Acceleration of vaginal patency was observed at 60, 250, and 1000 mg/kg bw/day. TPP administration did affect body weight; there was also a significant reduction in body weight at the attainment of vaginal patency (75g, 75g, and 67g vs. 106g in the control group, respectively). Significant changes were observed in organ weights of liver, adrenal glands, uterus, and ovaries. There were no changes in pituitary or luminal fluid weights.

The study authors concluded that TPP "exhibited estrogenic effects" in the 60, 250, and 1000 mg/kg bw/day groups based on the early attainment of vaginal patency, early occurrence of the first oestrus and decreased mean ovary weights.

RAC noted that the results of 4 female pubertal assays (Knapp, 2009a and 2009b; Knapp, 2007a and 2007b) indicated some estrogenic activity of TPP leading to acceleration of vaginal patency starting at doses 50 – 60 mg kg/day, lower mean absolute ovary weight at a dose of 50 mg/kg bw/day, earlier first occurrence of oestrous, oestrous cycle disturbances and absence or reduction in the number of corpora lutea at 200 mg/kg bw/day. No test substance-related effects on mean serum E2, LH, T4 or TSH levels were observed at 10, 50 and 200 mg/kg bw/day. Systemic toxicity was reported at 200 and 800 mg/kg as shown by reduced body weight in females at 200mg/kg and lethality at 800 mg/kg.

In vitro Rat Prostate Androgen Receptor Competitive Binding Assay (Thomas *et al*., 2012a)

Objective: To evaluate the ability of TPP to inhibit the binding of a radiolabelled ligand (^{3}H-R1881) to the androgen receptor (AR; responsible for key steps in the development of male sexual characteristics).

Study design: 30 male SD Crl:CD rats were castrated approximately 24 h before euthanasia to allow the endogenous concentrations of DHT and testosterone (precursor of DHT) to diminish. Immediately following euthanasia, the ventral prostate was collected. The prostate tissue was pooled and homogenized, followed by centrifugation to collect the cytosolic fraction containing the AR. The protein concentration in the cytosol was quantified immediately following the cytosol preparation and again on each day of the assay to provide a relative estimate of the AR concentration. The effect of the varying test substance concentrations on R1881 binding was evaluated by measuring the amount of ligand displaced by increasing concentrations of the test substance. The AR binding assay was thus conducted over such a range of test substance concentrations that a dose responsive curve could be developed if R1881 binding was affected by the presence of the test substance.

Results from these experiments indicate that TPP binds to the AR active site in a competitive manner with R1881 and is considered as an AR binder according to the data interpretation criteria in the protocol and the EPA guidance document. The IC_{50}, i.e. the inhibitory concentration at which 50% of the radio-ligand was displaced by the test substance, was determined from the dose-response curve.

The Relative Binding Affinity (RBA) for the non-labelled R1881, a ligand used in the assay as positive control, and dexamethasone used as weak positive control were in agreement with test guideline, and were higher than the RBA of TPP and, but their specific values were not given. The RBA for TPP was 1.57×10^{-7} %.

RAC notes that TPP was shown to have AR binding properties (Thomas *et al*., 2012a), however its RBA was 6 orders of magnitude (one million times) lower in comparison with the positive control, which shows that TPP has a rather weak binding affinity to the AR.

In vitro rat uterine estrogen receptor competitive binding assay (supporting study, Klimisch score: 1; Thomas *et al.*, (2012b)

Objective: To evaluate the ability of TPP to inhibit the binding of a radio-labelled ligand, hexatritiated 17β-estradiol, to the estrogen receptor (ER).

Study design: 30 female SD Crl:CD rats were ovariectomized approximately 9 days before euthanasia and their uterine cytosol used in the test. The test and control concentrations were 0.1nM – 0.1mM. The ligand was ^{3}H-E2; 19-norethindrone was used as positive control while octyltriethoxylsilane was used as negative control. The test material was TPP.

Results from this experiment indicate that TPP is a possible ligand for the rat ER, and the mean response curve indicated that TPP was able to inhibit competitive ligand binding. Therefore, TPP is considered interactive with the ER. The mean inter-day IC50 was approximately 1100 nM, and the RBA (%) of TPP relative to the reference estradiol ligand was 0.11%.

RAC notes that the TPP was shown to have ER binding properties (Thomas *et al*., 2012b). However its RBA was 4 orders of magnitude (10000 times) lower in comparison with the reference compound – estradiol, but it was ca. 3 times higher than the ER binding affinity of 19-norethindrone (weak positive control), which indicates that the binding affinity of TPP to the ER is weak.

Reproductive toxicity studies with TPP-derived mixture (supporting studies)

Several studies were presented in which the test materials used were TPP-derived chemical mixtures containing TPP as an impurity.

Two Generation Oral (Gavage) Reproductive Toxicity Study in the Rat (Nemec *et al.,* 1995)

Study design: Test material: EC No 272-234-3 (6.7 wt% TPP); SD rats, males/females; exposure by oral gavage; doses of 0, 50, 300 & 1000 mg/kg bw/day (=0, 3.4, 20.1, 67 mg/kg bw/day of TPP).

Two-generation study in rat (Wood *et al.,* 2002)

Study design: Test material: EC No 415-930-6 (3.8 wt% TPP); SD rats, 28 animals/dose and sex, exposure by oral gavage, doses of 0, 50, 250 & 1000 mg/kg bw/day (=0, 1.9, 9.5, 38 mg/kg bw/day of TPP), in accordance with OECD TG 416 (Two-Generation Reproduction Toxicity Study).

Two-generation study in rat (Wood *et al*., 2003)

Study design: Test material: EC No. 430-180-1 (26 wt% TPP); SD rat, 28 animals per dose and sex, exposure by oral gavage, doses of 0, 5, 30 & 150 mg/kg bw/day (0, 1.3, 7.8, 39 mg/kg bw/day of TPP); Exposure: F0 dosed for a minimum of 10 weeks prior to mating and then to dose-matched mating pairs throughout the mating, gestation and lactation phases. F1 offspring from each dose group were selected to proceed into the main study (at weaning). F1 dosed for a minimum of 10 weeks. Following this dosing period, the main study F1 animals were paired within dosing groups and subsequently dosed throughout mating, gestation and lactation to yield the F2 litters.

One-generation study (Knapp *et al*., 2008)

Study design: Test material: EC No455-880-2 (2.5 wt% TPP), SD rats, 30 animals per sex and dose, exposure by oral gavage, doses of 0, 50, 170, and 500 mg/kg bw/day (0, 1.25, 4.25, and 12.5 mg/kg bw/day of TPP); Exposure: F0 males and females for 70 consecutive days.

90-day study in rat (Haas *et al.*, 2010)

EC No 272-234-3 (6.7 wt% TPP), SD rats, 90-day study (male & female), exposure by oral gavage, doses of 125, 250, 500, 1000 mg/kg bw/day (8.4, 16.7, 33.4, 67 mg/kg bw/day of TPP), in accordance with OECD TG 408 (Repeated Dose 90-day Oral Toxicity Study in Rodents).

Since the detailed composition of the mixtures used in the above studies and the purpose of investigating reproductive toxicity of these mixtures are unknown, RAC is of the opinion that the

results of these studies have very limited relevance for classification of TPP for reproductive toxicity, therefore these studies will not be considered for justification of harmonized classification for TPP or for justification of SCL for this substance.

Developmental toxicity

Schroeder, 1987: Prenatal developmental toxicity study in rats (OECD TG 414, GLP compliant), key study

Study design: SD female rats were exposed once daily to 0, 20, 100 or 300 mg/kg bw/day of TPP by oral gavage during days 6 - 15 of gestation; Foetuses were evaluated for external, visceral, and skeletal alterations. Due to excessive mortality, dams in an additional group (500 mg/kg bw/day) were sacrificed on day 20 of gestation. Their uterine contents were examined.

No treatment-attributed effects occurred at the dose levels that did not produce marked maternal toxicity. Maternal toxicity effects included reduced body weight gain and food consumption. The weight gain remained low during the post-dosing period, gestation day (GD) 16-20. Soft stool was also observed during and after the dosing period. No adverse effects were observed in animals of the 20 or 100 mg/kg bw/day exposure groups. There were no necropsy observations attributed to treatment.

At 300 mg/kg bw/day, developmental effects included an increase in resorption that resulted in a reduction in litter size. Growth retardation was evidenced by reduced mean fetal body weight and reduced ossification. Three foetuses from one high dose litter had cleft palates and two foetuses (from different litters) had similar digit reduction defects (i.e., ectrodactyly); however, the incidence of high dose foetuses with external malformations (4/214 (1.9%) foetuses) did not differ statistically from the control animals. No increase in visceral malformations or variations was observed in the high dose group. The incidence of malformations at 300 mg/kg bw/day was statistically higher than in control animals. The skeletal malformation observed with greatest frequency at the high dose was wavy rib. Although identified as a malformation, this observation is often considered a variation with evidence of postnatal repair (Carney & Kimmel, 2007). Additional skeletal alterations were curved scapula and/or scapular spine and abnormally shaped long bones (humerus, ulna, radius and femur), and a statistically significant increase in skeletal variations (primarily reduced ossification).

In the two-generation study (Edwards *et al*., 2012; for details see 'Adverse effects on sexual function and fertility'), the timing of sexual maturation was significantly altered in both the male and female offspring of the first generation in the 75 mg/kg bw/day exposure group. At 75 mg/kg bw/day, pup body weights were significantly reduced in the F2 litters on PND 1 and PND 7-21 and in F2a litters on PND 14 and 21 compared to controls. However, no reduction in F2 and F2a pup body weight was observed at15 mg/kg bw/day.

Statistically significantly delayed attainment of balano-preputial separation was noted in F1 males in the 75 mg/kg bw/day treatment group as compared to controls (47.1 days vs. 45.1 in controls) in the presence of statistically significantly lower mean body weight (226.4 g vs. 246.2 g). The study director attributed the delay in attainment of this developmental landmark to the test-substance related lower mean body weight. There was no association between delayed preputial separation and failure to sire a litter.

In females, vaginal patency occurred at a younger age (27.4 days vs. 32.4 days) and at a lower body weight (60.8 g vs. 112 g) compared to controls; both differences were statistically significant. The timing of sexual maturation is influenced both by hormonal and growth factors. In females, sexual maturation was accelerated, despite the reduced growth rate. In the opinion of the study director, male sexual maturation was delayed due to delayed overall growth. As a result of these alterations in the timing of sexual maturation in the F1 offspring, anogenital distance was measured in the F2 offspring on PND 1 and was evaluated as a function of the cube root of pup body weights. There were no differences in anogenital distance between the groups.

In the one-generation study (Knapp, 2006; for details see 'Adverse effects on sexual function and fertility'), pups with potential exposure during gestation and lactation that were maintained in the study after weaning without post-weaning dosing, had unaffected sexual maturation in the 5 and 25 mg/kg bw/day groups (no statistical evaluation of pups from the 125 mg/kg bw/day group due

to insufficient litters). Offspring at 25 mg/kg bw/day showed statistically significantly reduced body weight gain compared to controls between PND 4 and 21. Pup body weight gain was not statistically evaluated for the 125 mg/kg bw/day dose group due to the small sample size.

Table 7. One-generation study: findings in offspring *(taken from CLH report of SI Group-UK, Ltd on TPP)*

Observations	**Time point**	**Sex**	**Dose level** (mg/kg bw/day)			
			0	**5**	**25**	**125**
Signs of toxicity		M/F	-	-	✔	✔
Litter size (#)	Day 0	M/F	13.3	14.0	12.4	2.3**
Viability (%)	Day 0	M/F	96.6	98.7	93.7	55.6
	Day 0-1	M/F	99.7	98.7	100	100
	Day 1-4	M/F	99.3	95.6	99.4	100
	Day 4-21	M/F	98.2	98.9	98.4	100
Pup weight(g)	Day 1	M	7.1	7.1	7.2	7.9
		F	6.6	6.7	6.8	8.0
	Day 4	M	9.6	9.9	9.6	10.8
		F	9.1	9.3	9.1	11.1
	Day 7	M	15.9	16.1	14.7	14.1
		F	14.6	15.3	13.5	16.9
	Day 14	M	33.0	33.5	29.9**	22.5
		F	31.2	32.3	28.0**	29.3
	Day 21	M	52.5	53.0	47.5**	34.5
		F	49.4	50.8	44.8**	46.1
Balano-preputial separation (day)		M	43.2	42.9	44.6*	47.5
Balano-preputial separation (g)			230.1	226.0	229.1	205.7
Vaginal patency (day)		F	33.0	32.8	33.5	32.5
Vaginal patency (g)			115.1	116.0	110.2	110.6

Statistical significance: *p<0.05; **p<0.01

RAC noted that during PC for the SI Group-UK, Ltd dossier, one MSCA commented that the observed detrimental effect on pup growth in the two-generation study (*Edwards et al., 2012)* and in the one-generation study (Knapp, 2006) justified a classification for effects via lactation and thus the addition of H362. However in the opinion of RAC the observed effects did not meet the CLP classification criteria for effects on or via lactation. This classification can be assigned if results of one- or two-generation studies in animals provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or if absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk. Hence, although effects on pup development have been consequently observed in F1, F2 and F2a litters at 75 mg/kg bw/day and also in offspring of mothers exposed to 25 mg/kg bw/day in the one-generation study, the existing evidence is considered not to meet the classification criteria for effects on or via lactation.

RAC is of the opinion that the small reduction of litter size and of foetal body weight and single malformations occurring in 1-3 foetuses of 1-2 litters in the group of 23 litters of dams exposed to TPP at dose of 300 mg/kg bw/day are due to significant maternal toxicity. No developmental toxicity was seen in foetuses in the groups exposed at 20 and 100 mg/kg bw/day. RAC notes that TPP at 500mg/kg bw/day induced high maternal lethality and at 300 mg/kg bw/day had induced significant maternal toxicity leading to considerable reduction of the body weight gain during pregnancy (by ca. 30% from 153 g in control group to 107 g in the 300 mg/kg group). Maternal body weight gain during the time of exposure from GD 6 until 15 in the 300 mg/kg group was only to 38% of the control value (50 g in control group and 19 g in the 300 mg/kg group), which shows a 72% reduction in body weight gain during organogenesis. It is noted that maternal toxicity was greater than the observed foetal toxicity. Food consumption from GD 6 until GD 15 in the 300 mg/kg group amounted to 68 g corresponding to 78% of the food consumption in control group (87 g). Therefore the existing data do not warrant classification of TPP as a developmental toxicant.

Summary of the classification justification

Classification for Repr. 1B, H360F according to the CLP Regulation, and Repr. Cat. 2: R60 according to DSD is supported when there is clear evidence from animal studies of an adverse effect of the substance on ***sexual function and fertility*** occurring together with other toxic effects, but where the adverse effects on fertility are not considered to be secondary non-specific consequences of other toxic effects.

Considering these criteria, classification as Repr. 1B; H360F (CLP) (Repr Cat. 2; R60, DSD) is justified for TPP based on the following effects observed in experimental studies:

- Reduced epididymal sperm count and prolongation of oestrous cycle at a dose of 75mg/kg in the two-generation reproductive study in rats (Edwards *et al.*, 2012).
- Reduced number of pups born in the F2a generation exposed to a dose of 75mg/kg (Edwards *et al.*, 2012).
- Reduced proportion of animals copulating when cohabited, reduced litter size, alterations in number of corpora lutea, prolongation of oestrous cycle and reduced epididymal sperm count in animals exposed at 125 mg/kg in the one-generation study in rats (Knapp, 2006).
- Acceleration of sexual maturation in female animals that is reported in the two-generation study and in the female pubertal assays.
- The mechanistic information further suggests that TPP has weak estrogenic and androgenic activity.

Impaired fertility has also been observed in the two-generation study in which a chemical mixture of unknown composition but containing TPP was given by gavage to rats at a dose of 67 mg TPP/kg bw/day (Nemec *et al*., 1995). The pregnancy index was reduced in the F0 and F1 generations in the two-generation study in which a preparation containing TPP was given by gavage to rats at a dose of 38 mg/kg bw/day (Wood *et al*., 2002). However, the unknown composition of the mixtures tested in these studies makes these results uncertain.

The effects observed in the two-generation and one-generation studies with TPP may be related to an estrogenic action of TPP which has been shown in uterotrophic bioassays in rats (Edwards *et al*., 2010a and 2010b), and in female pubertal assays in rats (Knapp, 2007a, 2007b, 2009a and 2009b). TPP is also considered as a substance interacting with the ER based on results of the *in vitro* rat uterine estrogen receptor competitive binding assay (Thomas *et al*., 2012b). Based on the *in vitro* rat prostate androgen receptor competitive binding assay (Thomas *et al*., 2012a) TPP is considered an AR binder. The binding affinity of TPP was similar to the weak positive control, dexamethasone.

Calculation of a concentration limit for reproductive toxicity

A proposal for the setting of an SCL for TPP was made by Chevron Oronite SAS, but it was not calculated according to the Guidance on the Application of the CLP Criteria. RAC therefore recalculated the proposed concentration limit in accordance with this guidance (version 3.0 – November 2012; point 3.7.2.5); see below.

Determination of the ED_{10} using the available data
The available data from animal studies were evaluated to establish the reproductive toxicity dose descriptor, ED_{10} (effective dose with a 10% effect level above the background/control group), as described below.

ED_{10} based on a 10% reduction in pups body weight

Table 8. Data from the two-generation study (Edwards et al., 2012)

Offspring of F0 females	Dose level (mg/kg bw/day)			
	0	1.5	15	75
Pup Weight (M/F) – PND 7	16.5/15.7	16.1/15.3	15.1*/14.4**	14.3**/13.5**
Pup Weight (M/F) – PND 14	30.1/29.2	30.1/29.1	28.6/27.3	22.9**/21.8**
Pup Weight (M/F) – PND 21	50.7/49.3	49.1/46.8	47.0/45.0*	36.4**/34.4**

*Statistical significance: *p<0.05; **p<.001*

In the two-generation study by Edwards *et al*. (2012) TPP induced reduction in the body weight of pups during lactation. A 10% reduction, compared to controls (16.5 g), of body weight of male pups on PND 7 gives a value of 14.85 g. Interpolation between 15 and 75 mg/kg bw/day to a dose level which would be expected to result in a male pup body weight of 14.85 g gives a value of 33.75 mg/kg bw/day.

(Calculations: (75 – 15)/(15.1-14.3) = 60/0.8= 75; 15.1 – 14.85= 0.25; 0.25 x 75 = 18.75; 15 + 18.75 = 33.75 mg/kg bw/day)

Female pups on PND 21:
A 10% reduction compared to the control body weight (49.3 g) of female pups on PND 21 gives a value of 44.37 g. Interpolation between 15 and 75 mg/kg bw/day to a dose level which would be expected to result in a male pup body weight of 44.37 g gives a value of 18.57 mg/kg bw/day. (Calculations: (75 – 15)/(45.0 - 34.4) = 60/10.6 = 5.66; 45.0 – 44.37 = 0.63; 0.63 x 5.66 = 3.57; 15 + 3.57 = 18.57 mg/kg bw/day).

ED_{10} based on 10% reduction in ovary weight

Table 9. Data from the one-generation study (Knapp *et al*., 2005)

Organ/tissue	Weight	Dose level (mg/kg bw/day)			
		0	5	25	125
Ovaries	(g)	0.1438	0.1417	0.1256*	0.1004*
	(%)	0.041	0.042	0.037	0.035**
	(g/100 g brain)	7.38	7.19	6.48**	5.20**

*Statistical significance: *p<0.05; **p<.001*

A 10% reduction of ovary weight compared to control females (0.144 g) gives a value of 0.130 g. Interpolation between 5 and 25 mg/kg bw/day to a dose level which would be expected to result in ovary weights of 0.130 g gives a value of 21 mg/kg bw/day.

(Calculations: (25 – 5)/(0.142– 0.127) = 20/0.015 = 1333; 0.142 – 0.130 = 0.012; 0.012 x 1333 = 16; 5 + 16 = 21 mg/kg bw/day)

ED_{10} based on 10% reduction in seminal vesicles weight

Table 10. Data from the one-generation study (Knapp *et al*., 2005)

Organ/tissue	Weight	Dose level (mg/kg bw/day)			
		0	5	25	125
Seminal vesicle	(g)	2.49	2.20**	2.10**	1.39**

*Statistical significance: *p<0.05; **p<.001*

A 10% reduction of the seminal vesicles weight compared to that of control males (2.49 g) gives a value of 2.24 g. Interpolation between 0 and 5 mg/kg bw/day to a dose level which would be expected to result in seminal vesicles weight of 2.24 g gives a value of 4.3 mg/kg bw/day.

(Calculations: (5 – 0)/(2.49 – 2.20) = 5/0.29 = 17.2; 2.49 – 2.24 = 0.25; 0.25 x 17.2 = 4.3; 0 + 4.3 = 4.3 mg/kg bw/day)

Based on the above data and in line with the criteria given in table 3.7.2.5.4 of the Guidance on the Application of the CLP Criteria, preliminary assignment of TPP was to the medium potency group, because its lowest ED_{10} (4.3 mg/kg bw/day) in rats is within the limits of this potency group (4 – 400 mg/kg bw/day). The other ED_{10}-values calculated also fall within these limits.

Modifying factors

In the guidance (point 3.7.2.5.5 of the Guidance on the Application of CLP criteria), it is stated that other factors, so called modifying factors, should be taken into account to establish whether the preliminary calculated potency needs to be modified. These factors, and the conclusion on each of them with regards to the potency of TPP, are presented one-by-one below.

Type of effect/severity (point 3.7.2.5.5.1 of the Guidance on the Application of CLP criteria)

The effects of TPP on fertility and sexual function in rats are not considered to be of very high severity, something that could potentially move the substance into a higher potency group, because even at doses inducing marked parental toxicity or repeated dose toxicity the effect on fertility was moderate, and was expressed mostly as a reduction in the weight of ovaries or male accessory sex organs and mild alterations of the oestrous cycle. Hence, TPP need not be moved to another potency group based on this modifying factor.

Data availability (point 3.7.2.5.5.2 of the Guidance on the Application of CLP criteria)

The data on reproductive toxicity of TPP are based on one- and two-generations studies, a prenatal toxicity study and repeated dose toxicity studies relevant for assessment of effects on sex organs, therefore there is no need to modify the assessment of potency due to limited data availability.

Dose-response relationship (point 3.7.2.5.5.3 of the Guidance on the Application of CLP criteria)

The findings from studies used for assessment of reproductive toxicity show a clear dose-response relationship, with some effects observed only at the highest used dose, e.g., reduced fertility in the one-generation study. TPP, based on LOAELs and on ED_{10}, should be assigned to the medium potency group, since there were no effects observed below 5mg/kg bw/day in one- or two-generation studies, while in repeated dose toxicity studies the NOAEL/LOAEL were at the level of 100 mg/kg bw/day, thus higher than the lower limit of 4 mg/kg bw/day and lower than 400 mg/kg bw/day, which is the upper limit of the medium potency group.

Mode or mechanism of action (point 3.7.2.5.5.4 of the Guidance on the Application of CLP criteria)

The mechanistic studies indicate that TPP has weak estrogenic activity, and may have a weak anti-androgenic effect. However, the adverse effects of TPP on fertility and sexual function in rats, which might be mediated by these mechanisms, are seen at dose levels also inducing general toxicity with reduced body weight and feed consumption.

Therefore, in the opinion of RAC there is no need to move TPP to another potency groups based on its estrogenic or anti-androgenic activity.

Toxicokinetics (point 3.7.2.5.5.5 of the Guidance on the Application of CLP criteria)

There are no data which would allow comparison of TPP toxicokinetics between rats and humans; therefore it will not influence its assignment to the medium potency group.

Conclusion

Conclusion on classification

RAC concluded that TPP fulfils the criteria for classification as Repr. 1B, H360F according to the CLP Regulation and as Repr. Cat. 2; R60 according to the DSD.

RAC further concluded that the existing data did not warrant classification of TPP as a developmental toxicant or classification for effects on or via lactation.

Conclusion on concentration limits

For medium potency substances the Guidance on the Application of the CLP criteria set 0.3% as the concentration limit (i.e. the general concentration limit according to the CLP criteria applies) for reproductive substances classified as Repr. 1B, and hence, based on that any preparation containing TPP at concentration equal to or in excess of 0.3% shall be classified with respect to reproductive toxicity, as Repr. 1B – H360F.

ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by the RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and rapporteurs' comments (excl. confidential information).