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-0000003060-91-04/F

Adopted

5 December 2013

5 December 2013

CLH-O-0000003060-91-04/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:

Chemical 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 number: 121158-58-5 [1]; 74499-35-7 [2]

The proposal was submitted by **SI group-UK, Ltd** and received by the RAC on **18 December 2012.** 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

SI group-UK, Ltd 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 **18 December 2012**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **1 February 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 that **Phenol, dodecyl-, branched [1]; Phenol, 2-dodecyl-, branched; Phenol, 3-dodecyl-, branched; Phenol, 4-dodecyl-, branched; Phenol, (tetrapropenyl) derivatives [2]** 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 statemen t Code(s)	
Current Annex VI entry	-	-	-	-	-	-	-	-	-	-
Dossier submitters proposal	-	Phenol, dodecyl-, branched [Tetrapropenylphenol (TPP)]	310-154-3	121158-58-5	Repr. 2 Skin Irrit. 2 Eye Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	H361f H315 H319 H400 H410	GHS07 GHS08 GHS09 Wng	H361f H315 H319 H410	-	M=10
RAC opinion	604-092-00-9	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[1] Aquatic Acute 1 Aquatic Chronic 1	H360F H314 H400 H410	GHS05 GHS08 GHS09 Dgr	H360F H314 H410	-	M=10 M=10

Resulting Annex VI entry if agreed by COM	604-092-00-9	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[2] Aquatic Acute 1 Aquatic Chronic 1	H360F H314 H400 H410	GHS05 GHS08 GHS09 Dgr	H360F H314 H410	-	M=10 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 (H 314: 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).

[2] see Footnote 1

Classification and labelling in accordance with DSD

	Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits
Current Annex VI entry	-	-	-	-	-	-	-
Dossier submitters proposal	-	Phenol, dodecyl-, branched [Tetrapropenylphenol (TPP)]	310-154-3	121158-58-5	Repr. Cat. 3; R62 Xi; R36/R38 N; R50-53	Xn; N R: 36/38-50/53-62 S: 26-36/37/39-60-61	N; R50-53: C ≥ 25 % N; R51-53: 2.5% ≤ C < 25% R52-53: 0.25 % ≤ C < 2.5 %
RAC opinion	604-092-00-9	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	604-092-00-9	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)- substitutions. The alkyl substituent is primarily a 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.

Hazard classes:

RAC assessed only the hazard classes for which a justification for action needed at community level (Art. 36(3) CLP Regulation) was provided by the dossier submitter (DS):

- Reproductive toxicity
- Skin corrosion/irritation
- Eye corrosion/irritation
- Environmental hazards

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

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

Six skin irritation studies in rabbits were evaluated in the CLH dossier. One of these studies (Waid *et al.*, 1989) was considered to be a reliable key study, while the others were considered as supporting studies. All indicated a skin irritation potential of TPP, with erythema and oedema in all animals at each observation time, generally not recovering within 72 h.

1) In the Waid *et al.* (1989) dermal irritation study (comparable to OECD TG 404; conducted according to GLP; Klimisch reliability score: 1) six female rabbits received a single 4-h application of 0.5 ml TPP to the intact and shorn flank. Irritation was scored at 1, 24, 48 and 72 h after removal of the test material and at 7 and 14 days, using a modified Draize scoring method.

Table 1. Results of the dermal irritation study by Waid et al. (1989)

Irritation parameter	Time point	Mean score in 6 animals	Remarks
Erythema score	24 h	4	Eschar (in 6 rabbits)
	48 h	4	Eschar (in 6 rabbits)
	72 h	4	Eschar (in 6 rabbits), cracking (in 1 rabbit)

	7 days	4	Eschar, cracking, thin layer of light brown necrotic skin, thick light brown flakes-flaky (in all rabbits).
	14 days	**2.8**	Thin layer of light brown necrotic skin (in 1 rabbit), thick light brown flakes/flaky (in all rabbits), alopecia (in 3 rabbits), thin fur regrowth (in 4 rabbits), sloughing (in 1 rabbit).
Oedema score	24 h	3.4	Fully reversible within 7 days.
	48 h	2.6	
	72 h	2.8	
	7 days	0	
	14 days	0	

The test material caused well-defined to severe erythema with no to moderate oedema within 1 h after unwrapping. Irritation descended into severe erythema and eschar formation with well-defined to severe oedema at 24 through 72 h. Cracking was observed in one animal at 72 h. Severe erythema, eschar, cracking, a thin layer of light brown necrotic skin and thick light brown flakes were present on day 7 with slight oedema observed in one animal.

At 14 days, well-defined to severe erythema, with eschar, a thin layer of light brown necrotic skin, thick light brown flakes, sloughing, alopecia and thin fur regrowth were present. There were no consistent differences between intact and abraded skin.

Microscopic findings included acanthosis (epidermal hyperplasia), hyperkeratosis and subacute inflammation in all sites. Epidermal exudate was also observed. These findings are considered to be compound-related and indicate mild dermal irritation. The results of histopathological examinations indicate that macroscopically observed changes 14 days after exposure were related to inflammatory processes, but they do not exclude occurrence of necrosis on skin sites which were not examined histopathologically but were only observed macroscopically.

2) In Cavalli *et al.* (1968), a dermal irritation study (supporting study; according to guideline: Federal hazardous substances act methods, 21 CFR; not GLP; Klimisch score: 2) six rabbits received a single 24-h (instead of 4-h) application of 0.5 ml TPP to intact and abraded skin. Irritation was scored at 24 and 72 h after removal of the test material and at 7 days, using the Draize scoring method.

Table 2. Result of the dermal irritation study by Cavalli et al. (1968)

Irritation parameter	Time point	Mean score of 6 rabbits	Max. score in individual rabbit	Reversibility	Remarks
Erythema score	24 h	2.3	3	Not fully reversible within 7 days	Intact and abraded skin
	72 h	4	4		
Oedema score	24 h	2.8	4		
	72 h	3	3		

There was severe irritation characterized by erythema and oedema. By 7 days, the skin was necrotic and lifting. Fur bearing skin was noted under the lifting area.

3) Another supporting study, Randall & Robinson, 1990, using six rabbits is also available for this endpoint. The study is considered to have a reliability Klimisch score of 2 as there is limited information available. The report is limited to a summary of the study and there is no individual animal data. The dermal exposure lasted for 24 h instead of 4 h as required in OECD TG 404. The test material used, Phenol, (tetrapropenyl) derivatives, has been assigned another CAS number

(CAS 27193-86-8) than the one used by the DS (121158-58-5). The purity was given as: ~93% p-dodecylphenol, contaminated with ~7% o-dodecylphenol. The resulting Primary irritation score was 8.0/8.0.

4) The Mürmann (1984) supporting skin irritation study was not considered as a key study as the 4-h exposed skin of rabbits (number of animals tested not known) was washed with warm water to remove the test material. Due to the low water solubility of the test material, it is unlikely that washing with water would be an effective means of removing the test material as specified in OECD TG 404. Therefore the exposure could have been longer than the reported 4 h in this study, and the study is considered invalid with a reliability rating of 3 according to the criteria of Klimisch. Only a summary of the study was made available.

Table 3. Results of the skin irritation study by Mürmann (1984)

Irritation parameter	Time point	Mean score	Reversibility
Erythema score	24 h	4	Not fully reversible within 72 h. Necrosis was reported to be present at study termination (day 6), however without providing any details concerning depth or extent of this change.
	48 h	4	
	72 h	4	
Oedema score	24 h	2.7	
	48 h	2	
	72 h	2	

5) The Mürmann (1988) supporting skin irritation study (number of animals tested not known) was not considered a key study as exposure was only for 3 minutes. In addition, as in the Mürmann, 1984, study, the exposed skin was washed with warm water to remove the test material and the exposure could therefore have been much longer than 3 minutes. The study is considered invalid by the DS with a reliability rating of 3 according to the criteria of Klimisch.

Table 4. Results of the skin irritation study by Mürmann (1988)

Irritation parameter	Time point	Mean score	Reversibility
Erythema score	24, 48 & 72 h	4	Not fully reversible within 72 h. Necrosis was reported to be present at study termination (day 5), however without providing any details concerning depth or extent of this change.
Oedema score	24, 48 & 72 h	4	

6) The Mürmann (1991) supporting skin irritation study was not considered a key study as exposure was only for 3 minutes and also in this study the exposed skin was washed with warm water to remove the test material. Therefore this study is considered invalid and a reliability rating of 3 was assigned according to the criteria of Klimisch.

Table 5. Results of the skin irritation study by Mürmann (1991)

Irritation parameter	Time point	Mean score	Reversibility
Erythema score	24, 48 & 72 h	4	Fully reversible within 17 days. At study termination (day 17) all skin injuries had healed with scar formation.
Oedema score	24, 48 & 72 h	4	

The DS concluded that TPP fulfils the criteria for Skin irritation classification according to both the CLP Regulation (Skin Irrit. 2; H315 – Causes skin irritation) and DSD (Xi; R36). The DSD classification was corrected to Xi; R38 (Irritating to skin) after the public consultation (PC; see below).

Comments received during public consultation

Two MSCAs provided support for the classification. One MSCA commented that the proposal should be Xi; R38 and not Xi; R36. The DS agreed that the classification should indeed be corrected to Xi; R38.

Assessment and comparison with the classification criteria

RAC took into account the following studies when deciding on the harmonised classification and labelling for skin corrosion/irritation:

The scores and skin observations in the Waid *et al*. study (1989) indicate that after a 4-h dermal exposure, TPP induced very severe skin inflammation lasting until the end of 14 days with signs of skin necrosis in some rabbits, but the necrosis was not confirmed in histopathological examinations. The 14-day observation period was too short for recovery of the skin damage.
In the Mürmann study (1984) skin necrosis was reported to be present at study termination (day 6 after exposure). The study had deviations from OECD TG 404 since after 4 h of rabbit skin exposure (number of animals tested not known) the skin was washed with warm water to remove the test material, which may not be sufficient to remove the substance due to its low water solubility.
In Cavalli *et al.* (1968), 7 days after 24-h dermal exposure, strong skin inflammation was observed and skin was reported to be necrotic in some rabbits.
In two rabbit studies with dermal exposure lasting only 3 minutes (the duration may have been longer due to insufficient removal of the substance from the skin) signs of necrosis was reported on day 5 after exposure in the first study (Mürmann, 1988) and in the second study all skin injuries had healed with scar formation at study termination (day 17; Mürmann, 1991)

In the opinion of RAC the results of these studies indicated that TPP caused skin necrosis in rabbits. However, due to poor study descriptions and the uncertain duration of exposure in some studies it was not possible to precisely determine the time of exposure leading to these effects. Using a weight of evidence approach and taking into account all available studies, it was concluded that TPP should be classified according to the CLP Regulation as Skin corrosive, subcategory 1C (for substances where responses occur after exposures between 1 and 4 h and an observation period up to 14 days) with a hazard statement H314: Causes severe skin burns and eye damage.

According to the DSD, TPP should be classified as Corrosive and assigned the risk phrase R34 (Causes burns) since based on results of the available studies it can be predicted that, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to 4 h exposure.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

Four eye irritation studies in rabbits were evaluated in the CLH report, all of them indicating an eye irritating potential of TPP. The eyes of the test animals developed conjunctival reactions that generally did not resolve within 72 h, and in some cases lasted up to 10 days after exposure. The DS concluded that the effects seen fulfil the criteria for classification as Eye Irrit. 2 – H319 (Causes serious eye irritation) according to the CLP Regulation, and Xi; R38 according to DSD. The DSD classification was corrected to Xi; R36 (Irritating to eyes) after the PC (see below).

1. In the Waid *et al*. (1990) eye irritation study (comparable to OECD TG 405; according to GLP; reliability - Klimisch score: 1), 0.1 ml of the test material was placed in the conjunctival sac of one eye in each of 9 rabbits. Three of the rabbits were further treated after a 30-second exposure by rinsing the eyes for 1 minute with distilled water at a rate of approximately 250 millilitres per minute. Eye irritation was scored at 1, 24, 48 and 72 h after exposure and at 7 and 14 days, using the Draize scoring method. According to the conclusion provided in the summary of the study

results, rinsing of eyes did not significantly affect the severity or persistence of irritation. Therefore, in the table below only effects in rabbits with un-rinsed eyes are presented.

Table 6. Results of the eye irritation study by Waid et al. (1990) – effects in rabbits with un-rinsed eyes

Irritation parameter	Time point	Mean score of 6 rabbits	Max. score of individual rabbits	Reversibility
Cornea opacity score	24, 48 & 72 h	0	-	Other: no effects seen
Iris lesion score	24, 48 & 72 h	0	-	
Conjunctivae redness score	24 h	1.8	3	Fully reversible within 10 days
	48 h	1.7	3	
	72 h	1.3	2	
Chemosis score	24 h	0.3	1	Fully reversible within 48 h
	48 h	0	-	
	72 h	0	-	

In treated, un-rinsed eyes, no corneal opacity or iritis was observed. The score for conjunctival redness was observed for 72 h after instillation, while chemosis disappeared after 24 h. Conjunctival redness continued to day 4 in rinsed eyes and day 7 in un-rinsed eyes. All eyes were clear by day 10. Alopecia and flaky skin were observed around the eye up to day 14.
Lackluster cornea, white material in the conjunctival sac, and a thickening of the conjunctival sac were also observed. Rinsing did not significantly affect the severity or persistence of irritation. The severity of the response did not meet CLP and DSD classification criteria for Eye irritation.

2. The Cavalli *et al.* study (1968) was not conducted according to a recognised guideline or GLP, although the methodology was basically similar to that described in the OECD TG. The study was considered to have a reliability rating of 2 according to the criteria of Klimisch by the DS. The reliability score of 2 was set as there was limited information on materials and methods and sufficient information was not provided on the reversibility of effects in the eyes (since observation ended 72 h after instillation of substance into the eyes).
0.1 ml of the test material was instilled into the conjunctival sac of the right eye of six rabbits. Observation and scoring of conjunctivitis, iritis, and corneal damage was done 24, 48, and 72 h after instillation.

Table 7. Results of the eye irritation study by Cavalli et al. (1968)

Irritation parameter	Time point	Mean score of 6 rabbits	Mean for 24, 48, and 72 h	Reversibility
Cornea opacity score	24 h	1.7	1.1 (effect only in 3 out of 6 rabbits)	Not fully reversible within 72 h.
	48 h	0.7		
	72 h	1		
Iris lesion score	24 h	0.5	0.5 (effect in 3 of 6 rabbits)	
	48 h	0.5		
	72 h	0.5		
Conjunctivae redness score	24 h	3	**2.7** (effect in 6 of 6 rabbits)	
	48 h	2.7		
	72 h	2.3		

Chemosis score	24 h	3.3	**3.3** (effect in 6 of 6 rabbits)	
	48 h	3.5		
	72 h	3		

The test material produced severe conjunctivitis (redness and oedema above the classification criteria cut-off) in all 6 test rabbits that lasted for 72 h after exposure, when observation was ended. The corneal opacity was very serious only in 3 rabbits with a score of 3, 4 and 3 after 24 h, 1, 3 and 0 after 48 h and 2, 4 and 0 after 72 h. There was no opacity of the cornea in the other 3 rabbits suggesting relatively high inter-individual variation in sensitivity. Thus the corneal opacity effect does not meet the classification criteria for Eye irritation according to either the CLP Regulation or the DSD.

The calculated mean values of the scores for redness & oedema of the conjunctivae (effects in 6 out of 6 animals) at 24, 48 and 72 h meet the classification criteria for Eye irritation according to both the CLP Regulation and the DSD.

3. The Randall & Robinson (1990) study is also available for this endpoint. The study is considered by the DS to have a reliability rating of 2 according to the criteria of Klimisch as there is limited information available and the report is only a summary of the study, lacking individual animal data.

Dodecylphenol (0.1 ml) was inserted into the conjunctival sac of 6 New Zealand male and female rabbits, which were then observed for 7 days.

The test material was moderately irritating to the rabbit eye, with a score of 33.3/110 according the scale rating of the Federal Hazardous Substances Act (21 CFR, § 191.12; 1964). Since grading of ocular lesions and particularly calculation of the mean score were not done according to EU regulations, classification according to the CLP or DSD criteria is not possible.

4. In the Mürmann, 1984 eye irritation study, no guideline was specified and the information was obtained from the peer reviewed, 2006 OECD SIDS dossier. Since the original report as well as individual animal data, are unavailable, classification of the substance according to the CLP or DSD criteria is not possible. This study is considered by the DS to have a reliability rating of 2 according to the criteria of Klimisch.

Application of 0.1 ml of the test material (97% purity) to the eyes of six rabbits caused slight to severe conjunctival irritation in all animals, which cleared within 72 h.

Table 8. Results of the eye irritation study by Mürmann (1984).

Irritation parameter	Time point	Mean Score	Max. score	Reversibility
Conjunctivae score (no specific information on redness or oedema was provided)	24 h	2.5		-
	48 h	2.2		-
	72 h	2		-

Five out of 6 animals had corneal opacity and iritis, and all but one of these animals was clear of irritation by 8 days. One animal had irritation that persisted for 21 days.

Comments received during public consultation

Several MSCAs provided support for the proposed classification. During the PC, one commenter said that the DSD classification should be revised to Xi; R36 and the DS agreed to this.

Assessment and comparison with the classification criteria

Taking into account the poor reporting and inconsistencies in results from the available studies, a weight of evidence approach was used, taking the Cavalli *et al*. study (1968), in which the effects were most pronounced, as the most informative study and the other studies as supportive.

Unfortunately, the reversibility of effects after 72 h after eye instillation was not followed in the Cavalli *et al.* study; thus reversibility is assessed based on all available studies.

Evaluation of intensity of effects:
In the studies where tests that have been conducted with more than 3 animals, such as in the Cavalli *et al.* study (1968), according to Guidance on the Application of the CLP Criteria (Version 3.0) classification as Eye irritation – Category 2 is justified when at least 4 out of 6 rabbits show:

a) mean score of ≥ 1 for the cornea opacity: this criterion is not met because the effects were seen only in 3 out of 6 animals;

b) mean score of ≥ 1 for the iris: this criterion is not met;

c) mean score ≥ 2 for conjunctival erythema: this criterion is met for the mean score at 24, 48 and 72 h in 6 out of 6 rabbits;

d) mean score ≥ 2 for conjunctival swelling: met for the mean score at 24, 48 and 72 h in 6 out of 6 rabbits.

Since TPP in the Cavalli study produced significant ocular lesions meeting the CLP classification criteria in 6 rabbits it should be classified as Eye Irrit. 2, with hazard statement - H319 (Causes serious eye irritation).

According to DSD, substances and preparations should be classified as Xi;R36 - Irritating to eyes, when it applied to the eye of the animal causes significant ocular lesions which occur within 72 h after exposure and which persist for at least 24 h:

a) corneal opacity equal to or greater than 2 but less than 3: this criterion is not met;
b) iris lesion equal to or greater than 1 but less than 2: this criterion is not met;
c) redness of the conjunctivae equal to or greater than 2,5: this criterion is met at 24, 48 and 72 h;
d) oedema of the conjunctivae (chemosis) equal to or greater than 2: this criterion is met at 24, 48 and 72 h.

Since the effects produced by TPP in properly performed test have met two of the above criteria it is concluded that TPP caused significant ocular lesions meeting DSD classification criteria and it should be classified, as Xi; R36 - Irritating to eyes

Evaluation of reversibility of eye effects:
In the reliable study of Waid *et al*., the eye changes were fully reversible within 10 days, although their degree of severity did not meet classification criteria.

The Cavalli *et al*. study does not report on reversibility since the last observation was made 72 h after eye instillation.

In the Mürmann study, the observed changes were reversible within 8 days after eye instillation in 5 out of 6 rabbits, and not clearly defined irritation signs were still observed in 1 rabbit. The study is poorly reported but it is interpreted that this unspecified effect in 1 out of 6 rabbits was not causing persistent damage which is required in order to consider this effect for classification.

Thus, the overall conclusion taking into account all studies is that it can be assumed that the eye effects are reversible, and there is not enough data to warrant classification in Eye Dam. 1; and therefore classification as Eye Irrit. 2 is more appropriate. However, since RAC concluded that the substance should be classified as Skin Corr. 1C with hazard statement H314 - Causes severe skin burns and eye damage, based on the assessment of skin irritation/corrosion effects, the classification as Eye Irrit. 2 is redundant and should not appear[1].

[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 (H 314: Causes severe skin burns and eye damage). Thus, in this case both classifications (Skin Corr. 1 and Eye

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The DS proposal to classify TPP for reproductive toxicity category 2 for adverse effects on sexual function and fertility was based on the following studies:

- two-generation study on rats (Edwards *et al*., 2010)
- one-generation study on rats (Knapp *et al*., 2005)
- relevant repeated toxicity studies:
    - 14-day rat gavage toxicity study (Harriman, 2003)
    - 28-day dietary rat toxicity study (Reyna & Thake, 1988)
    - 28-day gavage rat toxicity study (Harriman, 2004)
    - 90-day rat dietary toxicity study (Haas, 2007))
    - 90-day rat dietary toxicity study (Vogin, 1970)
    - 90-day dog dietary toxicity study (Vogin, 1970)

The assessment of developmental toxicity of TPP was based on the results of the prenatal toxicity study on rats with oral exposure by gavage (Schroeder, 1987).

The DS noted that TPP only affected sexual function and fertility at doses that also resulted in general systemic toxicity and concluded that adverse effects of TPP on reproductive function were not seen in a definitive two-generation dietary study. Deficiencies in the quality of the findings in rat gavage studies due to the likelihood of surpassing hepatic metabolic capacity as a result of oral bolus gavage dosing, and the lack of reproducible findings in another species (i.e. dog) questioned the relevance of the rat findings to humans. In this case a Category 2 classification would be more appropriate for TPP, which according to the DS would be consistent with previous RAC decisions based on similar data.

Based on a consideration of all of data, the DS proposed classification according to the CLP Regulation as Repr. 2 for fertility, and no classification for development or effects on or via lactation.

Comments received during public consultation

Four MSCAs commented that they do not agree with the proposal to classify TPP in Category 2 for fertility but that the data presented would support Category 1B. One of these MSCAs further said that the justification for the proposal was poorly argued, and one stated that in addition classification for developmental toxicity is justified.

Two MSCAs said that they agree with the proposed classification. However, one of these MSCAs commented that in addition to developmental toxicity, classification for effects on or via lactation is justified. The other MSCA recommended a better justification as to why the effects on development were not considered relevant for classification.

One MSCA commented that they agreed that the classification for TPP should be harmonised, but without further specifying whether or not they agreed with the proposed classification.

Five IND stakeholders commented that they supported the proposed classification as Category 2. One IND stakeholder (Chevron Oronite, SAS, DS of the other TPP CLH dossier) stated that they did not agree with Category 2 for fertility, but instead support Category 1B. They further submitted

---

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).

additional data, from studies not included in the SI group-UK, Ltd dossier, to support their statement.
One MSCA commented that the substance identity as well as the composition should be clarified.

Assessment and comparison with the classification criteria

RAC used a weight of evidence approach considering all data provided in both the CLH dossiers submitted for TPP (by the SI group-UK, Ltd and Chevron Oronite SAS, respectively) when concluding on the classification for reproductive toxicity of TPP.

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 the PC, the DS in response to a request from one MSCA, provided detailed results of this two-generation study, as 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 9. 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%)

Parameter F0 females and offspring	Dose Level (mg/kg bw/day)			
	0	1.5	15	75
Mean absolute organ weights and microscopic findings (incidences)				
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
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 10. 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.	40.9*/39.4**

Parameter F1 females and offspring	Dose Level (mg/kg bw/day)			
	0	1.5	15	75
Mean absolute organ weights and microscopic findings (incidences)				
			9	
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 11. 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 12. 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
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 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. As reported by the DS 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 13. One-generation study - findings in parental animals

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.

<u>90-day repeated dose toxicity study in rats (Haas, 2007)</u>
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.

<u>28-day repeated dose toxicity study in rats (Harriman, 2004)</u>
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.

<u>90-day study in rats with oral exposure in diet (Vogin, 1970a):</u>
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.

<u>90-day study in dogs with oral exposure in diet (Vogin, 1970b):</u>
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 a 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

In the opinion of the DS, the profile of male reproductive changes induced by TPP is consistent with the profile of changes reported for reproductive effects due to food restriction in male rats. 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 relatively 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/d 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/d, lower mean absolute ovary weight at a dose of 50 mg/kg bw/d, 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)

In the CLH report submitted by Chevron Oronite, SAS, several studies were presented in which the test materials used were TPP-derived chemical mixtures containing TPP as an impurity. More details on these studies can be found in the Background document and opinion for the TPP CLH dossier submitted by Chevron Oronite, SAS. Since the detailed composition of the mixtures used in these 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 a 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 14. 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, 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; see Chevron Oronite, SAS dossier). 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; see Chevron Oronite, SAS dossier). 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.

Conclusion:
RAC concludes 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 do not warrant classification of TPP as a developmental toxicant or classification for effects on or via lactation.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of Dossier submitter's proposal

The DS proposed to classify TPP as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) in accordance with the CLP Regulation, with an M-factor of 1 and 10, respectively. The corresponding classification according to the DSD is N; R50/53. The proposal was based on short-term algal toxicity (72-h E_rC_{50} of 0.36 mg/l) for acute and DSD classification, and a long-term invertebrate toxicity result (21-day NOEC of 0.0037 mg/l for *Daphnia magna*) for chronic classification under the CLP Regulation, together with the fact that the substance is not rapidly (or readily) biodegradable and has a fish bioconcentration factor (BCF) above 500 l/kg.

Comments received during public consultation

Several MSCAs pointed out that a more sensitive acute value is available for *Daphnia* (48-h EC_{50} of 0.037 mg/l), which affects the acute M-factor (changing it from 1 to 10). This was accepted by the DS.

One MSCA noted that the lack of ready biodegradation may have limited meaning for UVCB substances as the test cannot indicate whether all constituents are equally degraded. However, they supported the conclusion in view of the negative inherent study result.

The same MSCA suggested that the reported BCF values should be lipid normalized. In reply, the DS indicated that lipid normalized BCF values were not determined in the study. They stated that BCF values normalised to a 5% lipid content would be in the range 279 – 724 based on the lipid content of fish sampled on day 15 of the depuration period.

The same MSCA noted that aquatic toxicity results were reported in terms of nominal concentrations, but that none of the robust study summaries indicate whether test concentrations where adequately maintained. A previous regulatory report had recalculated the results from the key studies based on measured concentrations, and the MSCA thought that these should be taken into account.

The same MSCA indicated that the surrogate method should also be used for long-term hazard classification under the CLP Regulation, because reliable chronic data are not available for fish (although this does not lead to more stringent classification than that derived using chronic data for algae and aquatic invertebrates).

Finally, the same MSCA noted that branched nonylphenol (CAS no. 90481-04-2) is a constituent of the substance at a typical concentration of 0.6% (w/w) (range ≥ 0.5% to≤ 4.7%) and asked whether fish sex hormone disruption had been considered. The DS responded that the 48-h EC_{50} and 21-day NOEC for *Daphnia magna* are of a similar order of magnitude for both nonylphenol and dodecylphenol, so they consider that it can be largely excluded that toxicity is influenced by nonylphenol contained at low concentrations.

Assessment and comparison with the classification criteria

Degradability: TPP is not expected to hydrolyse under standard conditions at pH 4, 7 or 9. It failed a test for ready biodegradation (achieving at most 25% mineralisation in 28 days). An inherent biodegradability test also indicated a low degree of biodegradation (10% biodegradation after 56 days). Although the substance may contain some constituents that are more degradable than others, the available information indicates that it is neither rapidly degradable nor readily biodegradable in the environment.

Bioaccumulation: Measured fish BCF values from one study normalised to a 5% lipid content are in the range 279 – 724 (for edible and non-edible fish tissue) based on the lipid content of fish sampled on day 15 of the depuration period. RAC notes that information on fish growth and lipid content at different time points could have been considered for whole body BCF correction. However, in view of the conclusion on degradability, there is no need to investigate this further for classification purposes in RAC's view.

Table 15. Ecotoxicity: The lowest reliable ecotoxicity results were as follows (the key studies are highlighted in bold):

Trophic level	Species	Short-term result	Long-term result
Fish	*Pimephales promelas*	96-h LL_{50} = 40 mg/l	-
Aquatic invertebrates	*Daphnia magna*	48-h EC_{50} = 0.017 mg/l	21-day NOEC = 0.002 mg/l
Aquatic algae and plants	*Scenedesmus subspicatus* [*Desmodesmus subspicatus*]	72-h E_rC_{50} = 0.091 mg/l	72-h NOE_rC = 0.015 mg/l

The acute fish result is based on a water accommodated fraction derived from a nominal loading rate (no information is available about which constituents the fish were actually exposed to). The other results are based on mean measured concentrations as derived by Brooke *et al*. (2007). (These are slightly lower than the nominal values cited by the DS.)

Classification according to the CLP Regulation

Acute aquatic hazard: The lowest reliable short-term aquatic toxicity result is a 48-h EC_{50} of 0.017 mg/l for the cladoceran *Daphnia magna*. This is supported by acute toxicity data on algae. TPP should therefore be classified as Aquatic Acute 1 (H400), with an M-factor of 10 ($0.01 < L(E)C_{50} < 0.1$ mg/l).

Chronic aquatic hazard: The substance is not considered to be rapidly degradable. Reliable and relevant long-term aquatic toxicity data are only available for the invertebrate and aquatic algae/plant trophic levels. The lowest value is for *D. magna*, with a 21-day NOEC of 0.002 mg/l. Algae are around an order of magnitude less sensitive. These concentrations are below the threshold value of 1 mg/l for non-rapidly degradable substances, leading to classification as Aquatic Chronic 1 (H410) and an M factor of 10 ($0.001 < NOEC < 0.01$ mg/l). The surrogate approach needs to be considered for fish due to the lack of reliable long-term toxicity data: based on an acute 96-h LL_{50} of 40 mg/l combined with the substance's lack of rapid degradability, classification as Aquatic Chronic 3 (H412) would result. This is less stringent than the classification based on the *Daphnia* NOEC (and might not adequately reflect potential oestrogenic effects) and therefore is not relevant.

In summary, TPP should therefore be classified as Aquatic Chronic 1 (H400), with an M-factor of 10.

Classification according to DSD

The lack of ready biodegradation and 48-h EC_{50} of 0.017 mg/l for *D. magna* (with a similar value for algae) mean that TPP fulfils the criteria for classification with N; R50-53. The following specific concentration limits (SCLs) are applicable:

Table 16. Specific concentration limits and classification of the mixture

Concentration of branched 4-dodecylphenol in the mixture, C (w/w)	Classification of the mixture
$C \geq 2.5\%$	N; R50-53
$0.25\% \leq C < 2.5\%$	N; R51-53
$0.025\% \leq C < 0.25\%$	R52-53

In summary, the RAC agrees with the original proposal of the DS, although a more stringent acute M-factor (and SCLs under DSD) is necessary, based on a study that had been overlooked.

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).