Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the 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/A2

Adopted

5 December 2013

ANNEX 1 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON PHENOL, DODECYL-, BRANCHED; [1] PHENOL, (TETRAPROPENYL) DERIVATIVES;[2]

ANNEX 1 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON PHENOL, DODECYL-, BRANCHED; [1] PHENOL, (TETRAPROPENYL) DERIVATIVES;[2]

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

ECHA has compiled the comments received via the internet that refer to several hazard classes and entered them under each of the relevant categories/headings as comprehensively as possible. Please note that some of the comments might occur under several headings, when splitting the information provided is not reasonable.

ECHA accepts no responsibility or liability for the content of this table.

Last data extracted on 04.02.2013

Substance name: Phenol, dodecyl-, branched; [1] Phenol, (tetrapropenyl) derivatives;[2]
EC number: 121158-58-5[1]74499-35-7%20[2]
CAS number: 310-154-3[1]

Dossier submitter: SI

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	France		MemberState	1
Comment received				
We do not agree with the classification proposal.				
Dossier Submitter's Response				
This is responded to in the particular section detailing the comments received (see comment number 17).				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	Belgium		MemberState	2
Comment received				
We support the proposal to classify phenol, dodecyl-, branched for skin irritation 2 – H315, eye irritation 2 – H319. Wording remarks regarding the studies: o In the study Haas, M. C. : page 45 study of 2011 whereas page 48 and 64 it's 2007. o In the study Knapp JF : page 56 study of 2006 whereas page 68 it's 2005. o In the study Vogin (1970a) : it's indicated a NOEL of 25 mg/kg diet while the results mention an effect of the test material at highest dose level of 200 mg/kg and don't indicate effects at 100 mk/kg. Do you have any findings observed at this dose?				
Dossier Submitter's Response				
We appreciate the support of the irritation classification The Haas, M.C. 90-d repeat dose toxicity study was indeed completed in 2011. The Knapp JF, one-generation reproduction toxicity study was indeed completed in 2006. In the Vogin (1970a) 90-d repeat dose study in rats, testicular hypospermia occurred in 1/20 animals (control group), 0/20 (25 mg/kg), 2/20 (100 mg/kg) and 6/20 (200 mg/kg dose group). Statistically-significant reductions in relative testes weight was also evident at the high dose. In the absence of any adverse effect on testes weight among males dosed at				

100 mg/kg/day the marginally higher incidence of hypospermia at this dose level compared with controls was considered by the study director not to be substance related.
RAC's response
Thank you for your comment. In the opinion of RAC, the results of the studies indicate that TPP causes skin necrosis in rabbits; however, due to poor description and uncertain duration of exposure in some studies it is not possible to precisely determine the time of exposure leading to these effects. Using a weight of evidence approach with all available studies it is concluded that TPP should be classified according to CLP legislation as Corrosive in subcategory 1C (for substances where responses occur after exposures between 1 hour and 4 hours and observations up to 14 days) with the hazard statement H314: Causes severe skin burns and eye damage.

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	United Kingdom		Company-Importer	3
Comment received				
For this substance "Phenol, dodecyl-, branched; [1] Phenol, (tetrapropenyl) derivatives" EC 310-154-3 our company is aware that in addition to this harmonisation proposal for Reprotoxicity classification to Cat 2 there is another submitted proposal for harmonisation of Reprotoxicity classification to Cat 1b. To aid more efficient use of resources, to reduce uncertainty within the supply chain and to avoid duplication of effort (for ECHA, the RAC and industry) we urge ECHA to consider both submission proposals simultaneously and to use all the available data to conclude in one process a final determination of the Reprotoxicity classification.				
Dossier Submitter's Response				
The CLH dossier submitted by SI Group corresponds to the classification proposed in the joint registration dossier and was supported by the majority of the members of the SIEF. After our own submission, a further CLH dossier was submitted on the same substance by a company who disagreed with the classification agreed by the rest of the SIEF members. The second dossier currently undergoes public consultation in addition. We certainly welcome that both dossiers are dealt with in parallel resulting in a harmonised classification of the substance and removing uncertainty from the supply chain.				
RAC's response				
Both CLH dossiers were discussed and concluded in parallel by RAC.				

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	France	Total	BehalfOfAnOrganisation	4
Comment received				
Currently there are some discussions between our suppliers on the classification Cat 1B (H360) or Cat 2 (H361) for this endpoint. Their interpretations on the study's findings and in the CLP criteria leave one doubt on the TPP classification for the reproductive toxicity. One of the relevant findings from this report is the interpretation on the fact that toxicity on organs such as testes seems to be based on systemic toxicity and not on specific reproductive toxicity issue. This assumption is on line with supplier interpretations and ours. CLP criteria introduce some difference on the reproductive toxicity classification in comparison with DSD criteria. So, the section 4.11.5 is really useful and helps us to better understand how to classify substance for this endpoint. Therefore we support the Cat2 (H361) classification.				

Dossier Submitter's Response
We appreciate the support of the classification.
RAC's response
The option of classification of TPP as Repr. 2 was considered by RAC; however, classification as Repr. 1B was considered justified based on the information provided to RAC. The rationale and justification for the classification as Repr. 1B is contained in the opinion document.

Date	Country	Organisation	Type of Organisation	Comment number
30.01.2013	Netherlands		MemberState	5
Comment received				
According to part 1.1 of the CLH proposal, this substance can be identified by several different CAS numbers of which some are used according to the C&L inventory. It should therefore be considered whether the classification finally advised by RAC and included in an ATP by the commission should also include the other CAS numbers. If possible, please confirm the typical concentration or ranges of the constituents of the test substance used to carry out the human health and environmental studies. The concentrations should be comparable to those reported in the CLP Report under table 6 (page 22 of Part B). Page 21: The dossier submitter lists alternatives identifiers and names which describe the same substance. Nonetheless, we are wondering if they all have the same composition as that of phenol, dodecyl-, branched reported in the CLH. Specific comments - p60, Table 27: Please provide the number of animals per group. - p61, Table 28: Please provide a clear description/foot note of the data in the table, including an explanation for NE and for the values and values between brackets. - p63, table 29: epididymides weight (g) at dose level 20 mg/kg bw/d is 10.9. Please check this value.				
Dossier Submitter's Response				
Historically, several CASRNs have been used for this substance intermittently. To the best of our knowledge, linear dodecylphenol has never been of any commercial importance and is unlikely to have ever been supplied in any significant volume. Commercially, this substance is produced via an acid-catalyzed alkylation of phenol with a complex propylene tetramer feedstock. The feedstock contains a range of alkyl chain lengths and branching patterns. In addition to variations in alkyl chain length, extensive branching also occurs during alkylation process. The commercial product is Phenol, alkylation products with C10 - 15+ branched olefins from propylene tetramer manufacture, mainly para-substituted, UVCB. The C12 moiety predominates, but only occasionally at 80%. No other carbon number homologue, e.g. C11 or C13, is consistently above 10% (therefore not multi-constituent) although they may be present >10% for specific samples. Phenol, dodecyl-, branched (EC 310-154-3 / 121158-58-5) is the only CASRN that has been registered under REACH so far. The substance is alternatively also known as tetrapropenylphenol (TPP); this nomenclature is a consequence of the production of the substance via the alkylation of phenol with propylene tetramer. Composition of the test substances Specifically for the older studies, the detailed composition of the test sample used has not				

been described in detail in the respective study reports and therefore can no longer be totally reconstructed. Nevertheless, it is reasonable to assume that these samples would correspond to typical commercial qualities of the substance that were on the market at the time the studies were conducted. Such composition has been summarized in the below table. The more recent studies (i.e. bioconcentration, chronic daphnia, 1- and 2-generation reproduction toxicity, 90-day repeat dose toxicity) were all conducted with a fully characterized substance sample. This is given in the below table as typical values, as confirmed with the company who had provided these samples:

Specific Distribution Of TPP Homologs (wt, percents)

Homolog	Typ.	Min.	Max.	CASRN	EC No.
Phenol, C5-8, branched	4.7	2	6.0	Ukn.	Ukn.
Phenol, C9, branched	1.3	0.5	2.0	90481-04-2	291-844-0
Phenol, C10, branched	3.2	2.0	7.0	Ukn.	Ukn.
Phenol, C11, branched	14.6	10.0	20.0	Ukn.	Ukn.
Phenol, C12, branched	45.4	40.0	60.0	121158-58-5	310-154-3
Phenol, C13, branched	14.4	10.0	20.0	Ukn.	Ukn.
Phenol, C14, branched	8.0	5.0	15.0	Ukn.	Ukn.
Phenol, C15, branched	5.3	3.0	8.0	Ukn.	Ukn.
Phenol, C15+, branched	3.1	1.0	5.0	Ukn.	Ukn.

Empirical Formula for TPP (C10-15 range): $C_{16\text{-}21}H_{26\text{-}36}O$

Page 60 table 27 relating to the study (Reyna & Thake, 1988; Monsanto ML-87-041)
The substance (TPP) was evaluated in a 28-day oral feeding study in rats (OECD Guideline 407). It was administered by the oral route in the diet to 10 animals per sex at each dose level at concentrations of 0, 500, 2500, and 5000 ppm (approximately 0, 25, 125, and 250 mg/kg/day) for 28 consecutive days.

Page 61 relating to the study (Reyna & Thake, 1988; Monsanto ML-87-041)

Revised Table 28: 28-day rat gavage study: summary of pathology

Shading illustrates the doses of TPP that resulted in evidence of both systemic toxicity and effects on organs and tissues including the male reproductive tract.

Observation	Sex	Dose level (mg/kg bw/d)			
		0	40 (500 ppm)	180 (2500 ppm)	300 (5000 ppm)
Liver – periportal vacuolization	M	-	NE	NE	3 (1-2)
	F	-	NE	NE	1 (1)
Kidney – mineralization	M	1 (1)	NE	NE	2 (1)
	F	2 (1)	NE	NE	1 (1)
Bone Marrow – hypoplasia	M	-	-	-	6 (1)
	F	-	-	-	3 (1)
Spleen – congestion	M	-	-	1 (1)	4 (1)
	F	-	-	-	5 (1)
Testes – tubular hypoplasia	M	-	-	-	4 (3-5)
Epidydimides – decreased or absent sperm	M	1 (5)	-	-	4 (4-5)
Epidydimides – hypoplasia	M	-	-	-	1 (4)

Seminal Vesicles – absence of secretions	**M**	-	-	-	5 (5)
Prostate – abnormal or absent secretions	**M**	-	-	-	7 (2-5)
Prostate – hypoplasia	**M**	1 (3)	-	-	4 (3-5)

 # = number of animals.
NE: not examined
( ) = severity of microscopic changes: 1 = minimal; 2 = mild; 3 = moderate; 4 = severe

Page 63 table 29 relating to the 28-day gavage rat toxicity study [Harriman, 2004; WILResearch Laboratories, WIL-186032] contains a typographical error Epididymides weight at the dose level 20 mg/kg bw/d is 1.09 (g).

RAC's response

Thank you for your comment.

Date	Country	Organisation	Type of Organisation	Comment number
18.01.2013	Germany		Company-Manufacturer	6

Comment received

We agree with the suggested classification. The data clearly demonstrate irritating properties, both for skin and eyes, but lack of mutagenicity, carcinogenicity etc. The most critical endpoint is reproductive toxicity. Further specific comments are provided below. We also agree with the evaluation that the substance is toxic to aquatic organisms, both after acute and chronic exposure.

Dossier Submitter's Response

We appreciate the support of the classification.

RAC's response

In the opinion of RAC, taking into account all studies, it is assumed that the eye effects are reversible, and there is not enough data to warrant classification in Eye Dam. 1, therefore classification Eye Irrit. 2 was considered more appropriate. However, since RAC also concluded on classification as Skin Corr. 1C with hazard statement H314: Causes severe skin burns and eye damage based on skin irritation/corrosion assessment, the classification Eye Irrit. 2 will not be included in Table 3.1, Annex VI to CLP. For further information, see Note 1 to the Classification table in the opinion document.

Date	Country	Organisation	Type of Organisation	Comment number
29.01.2013	United Kingdom		Company-Downstream user	7

Comment received

My Company supports the conclusions of the dossier and proposed harmonized classifications. RAC needs to ensure consistency with previous RAC opinions on the classification for reproductive toxicity and Category 2 for reprotoxicity would appear to be consistent. We do have concerns over the criteria for applying these reproductive classifications being not sufficiently well defined and potentially allow different conclusions to be drawn. Additional guidance for this end point would be welcome.

Dossier Submitter's Response

No further comments.

RAC's response
The option of classification of TPP as Repr. 2 was considered by RAC; however, classification as Repr. 1B was considered justified based on the information provided to RAC. The rationale and justification for the classification as Repr. 1B is contained in the opinion document.

Date	Country	Organisation	Type of Organisation	Comment number
29.01.2013	Finland		MemberState	8
Comment received				
We agree with the dossier submitter that there is a need for a harmonised classification for Phenol, dodecyl-, branched.				
Dossier Submitter's Response				
No further comments.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
28.01.2013	Norway		MemberState	9
Comment received				
CLH report for Phenol, dodecyl-, branched; [1] Phenol, (tetrapropenyl) derivatives; [2] - Comments from Norway Norway would like to thank for the industry proposal for harmonised classification and labeling of Phenol, dodecyl-, branched; [1] Phenol, (tetrapropenyl) derivatives; [2], CAS no 121158-58-5 [1] 74499-35-7 [2]. The proposal for cas number 121158-58-5 currently on public consultation suggest a classification with repr cat 2. We would like to draw the attention to the Registry of Intention (ROI) for classification and labeling. There seems to be another industry proposal for classification and labeling for cas number 121158-58-5 with submission date 15/11/2012 suggesting classification with repr cat 1B (http://www.echa.europa.eu/web/guest/registry-of-submitted-harmonised-classification-and-labelling-intentions/-/substance/1401/search/+/term). We do not know the background for the two different proposals, but it might be relevant for RAC to evaluate these two proposals together.				
Dossier Submitter's Response				
Please see the previous comments in respect to the other CLH submission for this substance (comment number 3)				
RAC's response				
There were indeed two submissions for harmonised classification of reproductive toxicity of Phenol, Dodecyl-, Branched. Both proposals were evaluated in parallel and two opinion development documents have been prepared, one for each submitted CLH dossier, but concluding on the same classification for Reproductive toxicity – Repr. 1B.				

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	United States	Chevron Oronite SAS	BehalfOfAnOrganisation	10

ANNEX 1 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON PHENOL, DODECYL-, BRANCHED; [1] PHENOL, (TETRAPROPENYL) DERIVATIVES;[2]

Comment received
Please see attached document.
Dossier Submitter's Response
Responded to in the particular section further below giving more details on the submitter comments (comment number 25)
RAC's response
The response is provided jointly with the response to comment No. 25 provided by Chevron Oronite SAS, France.

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Sweden		MemberState	11
Comment received				
The Swedish CA supports the proposed classification of Phenol, dodecyl-, branched / Phenol, (tetrapropenyl) derivatives (CAS nr 121158-58-5, 74499-35-7) as a reproductive toxicant in category 2 regarding effects on fertility. We also support the proposed classifications regarding skin (category 2) and eye irritation (category 2), as well as the ones proposed for hazards to the aquatic environment (aquatic acute category 1[M-factor 10] and aquatic chronic category 1 [M-factor 1]). In addition we propose that it also should be classified for developmental toxicity (category 2) and for effects via lactation. The rationales for the proposals are described below.				
Dossier Submitter's Response				
We appreciate the support of the fertility, irritation and the aquatic environment classification. Further comments on the further endpoints are provided in the particular sections below.				
RAC's response				
Thank you for your comment. In the opinion of RAC, classification as Repr. 1B, H360F is justified taking into account the clear evidence from animal studies on an adverse effect of TPP on sexual function and fertility. The effects occurred together with other toxic effects, but were not considered to be secondary non-specific consequences to other toxic effects. In the opinion of RAC, classification as Eye Irrit. 2 is more appropriate as suggested by you, based on the available data. However, since RAC also concluded on classification as Skin Corr. 1C with hazard statement H314: Causes severe skin burns and eye damage based on skin irritation/corrosion assessment, the classification Eye Irrit. 2 will not be included in Table 3.1, Annex VI to CLP. For further information, see Note 1 to the Classification table in the opinion document. Reproductive toxicity and aquatic toxicity have been evaluated and RAC's conclusion is included in the opinion documents. The developmental toxicity has been considered; however, the maternal toxicity seen was considered as being greater than the observed fetal toxicity. Therefore the existing data do not warrant classification of TPP as a developmental toxicant(see also RAC response to comment No 18). Classification for effects on or via lactation was not supported by RAC since the observed effects do not meet the CLP classification criteria for this category.				

Date	Country	Organisation	Type of Organisation	Comment

				number
31.01.2013	Germany		MemberState	12
Comment received				
Overall the German CA supports to harmonise classification and labelling for Phenol, dodecyl-, branched. Nevertheless on p.9 and p.17 should be noted that Precautionary Statement P337+P313 is missing (indicated by the results of the eye irritation section).				
Dossier Submitter's Response				
We agree with the comment.				
RAC's response				
Thank you for your comment. However, P-statements are not harmonised.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	Germany		MemberState	13
Comment received				
p.55: The statement "In the absence of any evidence of carcinogenicity in animal studies" seems to give an impression of a firm conclusion based on data. No animal study on carcinogenicity was conducted or exists. A more appropriate formulation is suggested: 'Conclusion on carcinogenicity is not possible due to the fact that no carcinogenicity study is available. Taking into account the absence of indications from other studies including mutagenicity studies the test substance does not fulfils the criteria for classification.'				
Dossier Submitter's Response				
We agree with the comment.				
RAC's response				
Agree.				

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	France		MemberState	14
Comment received				
P13: Absence of genotoxicity is not sufficient to conclude on carcinogenicity properties of the compound. Based on the available data, no conclusion on carcinogenicity is possible.				
Dossier Submitter's Response				
We agree with the comment.				
RAC's response				
Agree.				

Date	Country	Organisation	Type of Organisation	Comment number
29.01.2013	United Kingdom		Company-Downstream user	15
Comment received				
Whilst there is no data available, the lack of positive results in the in vitro and in vivo mutagenicty studies does not give cause for concern.				
Dossier Submitter's Response				
We agree with the comment.				
RAC's response				

Agree.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
29.01.2013	United Kingdom		Company-Downstream user	16
Comment received				
There is clear evidence from in vitro and in vivo mutagenicity studies to support no classification.				
Dossier Submitter's Response				
We agree with the comment.				
RAC's response				
Agree.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	France		MemberState	17
Comment received				
The relationship between systemic toxicity and the reproductive effects is doubtful. All studies in rats showed effects on sexual organs in males but cannot be explain only by systemic toxicity. Some findings in the reproductive studies (one and 2 generations) suggest an endocrine active effect. Justifications to propose only a category 2 for fertility are poorly argued: ☐ P68: suspicion of species-specific sensitivity of rats based on no effects observed on dogs. But in this study, only 3 dogs are tested and the maximal dose is 143 mg/kg/d. ☐ P84: poor reproductive performance and advanced age of animals. This argument should be justified by a bibliographic reference. In this context, a category IB (H360F) seems more appropriate. A comparison of reproductive toxicity with the other alkyl phenol might be appreciated.				
Dossier Submitter's Response				
We thank the French Competent Authority for their important comments. We agree that repeated-dose treatment of rats with the substance resulted in effects on some reproduction parameters in a dose-responsive fashion but, as the following demonstrates, the entire cohort of data does show a demonstrable, close relationship between the effects on reproduction and concomitant systemic toxicity: A collated review of the set of repeated-dose studies available for the substance illustrates the potential of this substance to induce a spectrum of generalised systemic toxicity: • 500 mg/kg/ day – mortality in addition to symptoms below • 300 mg/kg/ day – decreased hemoglobin, reduction in heart weight (absolute and vs brain), liver hypertrophy, adrenal hypertrophy, body weight (absolute and gain) reductions, reduced food consumption, reticulocyte reductions, red-colored urine • 200 mg/kg/day – reductions in red blood cell counts and haemoglobin, reductions in white blood cell and lymphocyte counts, adrenal hypertrophy, body weight reductions (absolute and gain), reduced food consumption and reduced food utilisation efficiency • 180 mg/kg/day – decreased haemoglobin, reduction in heart weight (absolute and vs				

brain), liver hypertrophy, adrenal hypertrophy, body weight (absolute and gain) reductions, reduced food consumption, reticulocyte reductions, red-colored urine
- 150 mg/kg/day – clinical observations of perianal staining, hair loss, soft feces, clear yellow/brown material on body surfaces, and unkept appearance, adrenal hypertrophy, periportal hepatocellular vacuolisation, body weight reductions (absolute and gain), reduced food consumption and reduced food utilisation efficiency
- 125 mg/kg/day – adrenal hypertrophy, chromodacryorrhea, body weight reductions (absolute and gain), reduced food consumption, reduced food utilisation efficiency
- 100 mg/kg/day – adrenal hypertrophy, reduced food consumption and reduced food utilisation efficiency, body weight reductions (absolute and gain)
- 75 mg/kg/day – adrenal hypertrophy, reductions in body weight (absolute and gain), reduced food consumption, reduced food utilisation efficiency
- 50 mg/kg/day – body weight reductions, reduction in food consumption, kidney effects
- 25 mg/kg/day - chromodacryorrhea, body weight reductions, reduced food consumption, reduced food utilisation efficiency

The multitude of findings over a broad range of doses clearly demonstrates that the substance imparts adverse systemic effects on the rat. Across all repeated-dose studies, effects on reproduction parameters were consistently associated with evidence of generalized systemic toxicity. This is exemplified by the clear differentiation in all studies between a) doses that result in systemic toxicity concomitant with effects on reproduction parameters, and b) doses that do not result in systemic toxicity or reproduction effects (please refer to shaded dose-observation relationships illustrated in Tables 26 through 45 of the CLH dossier).

This systemic toxicity relationship is further supported by the results from the 90-day dietary study where the substance was observed to cause significant reductions in body weight (absolute and gain) which *pre-emerged* (i.e. were evident at 50 mg/kg/day) before any effect was observed on reproduction parameters at the next higher dose (100 mg/kg/day). This finding suggests that the effects on reproduction parameters were secondary to systemic toxicity.

Furthermore, it is commonly recognised that in repeated-dose studies when systemic toxicity precedes a secondary effect on an organ or tissue that the cessation of treatment results in a reversal of that secondary effect. This is demonstrated in the 28-day gavage study with high-dose recovery groups (Harriman, 2003). At the end of the 14-day treatment recovery period systemic toxicity induced by the substance dissipates, followed by evidence of a similar recovery among reproduction parameters as well. This series of observations support the premise that the effects of the substance on reproduction parameters occur as a secondary response to generalized systemic toxicity, and thus is consistent with a decision to consider TPP as a suspected human reproductive toxicant CLP Category 2.

The observation that effects on reproductive endpoints in the male predominantly occurred in the presence of systemic toxicity has been recognised in the public consultation comments submitted by Competent Authorities from the Netherlands and Sweden, as well as responses from Chevron Oronite SAS, the submitter of the second dossier proposing a different classification. Additionally, the conclusion that effects on male reproduction parameters are secondary to systemic toxicity is exemplified by the lack of consistency in effects observed. Several examples are brought forward below:

1) A careful review of the data from the 2-generation study shows clear differences in the response of F0 and F1 males to treatment. The effect of 75 mg/kg/day TPP on testes weights shows a discrepancy for the right organ (a slight effect in the F0 males, but no effect in the F1 males) and in the left testes (no effect in the F0 group, yet a significant effect in the F1 group). A direct acting reproduction toxicant would

be expected to produce consistent effects between paired organs in treatment groups. The inconsistency in effects within the same study suggest that the effects on the male reproduction system is indirect and occurs secondary to systemic toxicity.

2) Whereas there was a reduction in absolute mean left epididymides in the F0 males within the 2-generation study, there was no reduction in the F1 males who were treated much longer at 75 mg/kg/day. In fact, at 15 mg/kg/day mean weights were actually significantly higher. Again, this finding demonstrates clear inconsistencies in the observed effect of treatment within the same study.
3) In the 1-generation oral gavage study, testes weights were reduced at 125 mg/kg/day contrary to findings in the 90-day dietary study where testes weights were increased at 100 and 150 mg/kg/day demonstrating an inconsistent effect among studies. It is our opinion that the absence of a consistent effect supports the conclusion that the observed effects on male reproductive organs represent a secondary response to generalized toxicity.

With regard to the dog study, an evaluation conducted by two independent toxicological consultants concludes that the results of the 90-day dog dietary study (Vogin 1970) are reliable and represent a valid assessment of the repeated dose toxicity of the substance. The highest dose tested in dogs (approximately 150 mg/kg/day) is comparable to doses that caused a spectrum of systemic toxicity in rats (i.e., decreased hemoglobin, reduction in heart weight (absolute and vs brain), liver hypertrophy with periportal hepatocellular vacuolization, adrenal hypertrophy, body weight reductions, reduced food consumption, clinical observations of perianal staining, red-colored urine, hair loss, soft feces, clear yellow/brown material on body surfaces (chromodacryorrhea) and unkept appearance over the course of a 90-day treatment period. Most significantly, under such a treatment regime there were no adverse effects on reproduction parameters in dogs.

The French Competent Authority raises a valid and interesting analogy with other alkyl phenols that have been evaluated for effects on reproduction. In fact, there is a high degree of structural similarity between nonylphenol and the substance (TPP). The toxicological similarity between these substances is established by reviewing data extracted from key endpoints published on the ECHA dissemination website. Nonylphenol has been observed in rat studies to cause effects on ovary weight, oestrus cycling, changes in time to vaginal opening, effects on accessory glands and minor perturbations in the reproduction system of offspring. A comparison of the findings from reproduction toxicity studies shows that treatment of rats with nonylphenol over 3 generations resulted in a reproduction NOAEL level = 15 mg/kg/day. Similarly, TPP in a 2-generation test yielded a NOAEL level = 15 mg/kg/day in the same species. There already exists a harmonised classification for nonylphenol as a suspected human reproductive toxicant, Category 2. Given the recognized high degree of similarity between nonylphenol (structural and toxicological) and TPP it is appropriate to presume that there will be a consistent approach to classification and this supports the current proposal to classify TPP as a suspected human reproductive toxicant, Category 2.

Finally, the conclusion in the definitive 2-generation dietary study that the reduced litter size observed in the F2a cohort at the highest dose tested was likely to be associated with the advanced age of the F1 parents is supported by data found in Palmer and Ulbrich, Fund Appl Toxicol, 38, 7-22, 1997.

RAC's response

Thank you for your comment. In the opinion of RAC, classification as Repr. 1B, H360F, is warranted. See also RAC response to comment No. 18.

It is possible that there are some toxicological similarities with other alkyl phenols, but that RAC has not reviewed nor compared the data with the CLP criteria for any of these.

Date	Country	Organisation	Type of Organisation	Comment

				number
31.01.2013	United Kingdom		MemberState	18

Comment received

Fertility

We do not agree with the proposed classification for fertility, in our opinion, classification with H360F is more appropriate, based on the findings of the 1-genration study (Knapp et al 2005).

From the 1-generation gavage study (Knapp et al 2005) reductions in fertility and effects on reproductive organs occurred at doses that also caused more general toxic effects (125 mg/kg/day the highest dose tested); including reduced bodyweight gain and food consumption and changes in the adrenals, kidneys and liver. However, this toxicity was not considered to be particularly severe such that the adverse effects on fertility could have been a secondary non-specific consequence of it.

The lack of clear adverse effects on fertility in a more recent 2-generation (Edwards et al 2010) dietary study at doses of up to 75 mg/kg/day does not provide sufficient reassurance that the earlier positive study can be discounted. It is possible that the negative findings might be related to toxicokinetic differences between gavage and dietary administration. However, it is also possible that sufficiently high doses were not used. We note that adverse effects on reproductive tissues, and general toxicity, were observed in the 90-day dietary study by Hass et al (2007) at doses of 150 mg/kg/day and above.

Developmental toxicity

We do not agree with the position that no classification is appropriate for developmental toxicity. In our opinion the findings of skeletal malformations and resorptions from the Schroeder study (1987) support classification. However, further discussion is needed in relation to the potential contribution of maternal toxicity and the severity of classification.

The results from the developmental toxicity study (Schroeder et al 1987) provide evidence of developmental toxicity at 300 mg/kg/day, the highest dose tested. At this dose the incidence of uterine resorptions was increased, and there was a significant reduction in mean litter size and foetal body weights. In addition, the incidence of total skeletal malformations at this dose level was significantly increased. Of particular note were long bone malformations, curved scapula and ectrodactyly (short or missing digits). These malformations are generally not considered to occur as a secondary non-specific consequence of maternal toxicity.

Dossier Submitter's Response

We thank the United Kingdom Competent Authority for their appropriate comments. We agree that treatment of rats with TPP in the 1-generation study resulted in effects on reproduction parameters in a dose-responsive fashion. However, adverse findings are only observed at doses that elicit manifestation of clear systemic toxicity. Evidence of generalised systemic toxicity was reported as: 125 mg/kg/day – adrenal hypertrophy, chromodacryorrhea, body weight reductions (absolute and gain), reduced food consumption, reduced food utilisation efficiency, and at 25 mg/kg/day - chromodacryorrhea, body weight reductions (absolute and gain), reduced food consumption, reduced food utilisation efficiency.
In the 1-generation study, as with all of the other repeated dose studies, there is a clear differentiation between doses that result in concomitant systemic toxicity and effect on reproduction parameters, and doses that do not result in systemic toxicity or reproduction

effects (please refer to shaded dose-observation relationships illustrated in Tables 26 through 45 of the CLH dossier).
This pragmatic and causal relationship should not be underestimated, and is further illustrated by the results from the 90-day dietary study where systemic toxicity resulted in significant reductions in body weight (absolute and gain) which pre-emerged before any effect on reproduction parameters (i.e. bodyweight effects were evident at 50 mg/kg/day whereas. Effects on reproduction parameters begin to manifest at the next higher dose at 100 mg/kg/day, secondarily to systemic toxicity). In most cases where repeated dosing of a substance causes generalised systemic toxicity which precedes an adverse effect on an organ or tissue, cessation of treatment results in resolution of that secondary organ effect. This is exactly what happened in the 28-day gavage study with high dose recovery groups (Harriman, 2003). In this study, at the end of the 14-day treatment recovery period systemic toxicity induced by the substance dissipates, followed by evidence of a similar recovery among reproduction parameters as well. These observations, together with the absence of any significant adverse effects observed in the absence of systemic toxicity all support the conclusion that the effects of TPP on reproduction parameters occur secondary to generalised systemic toxicity and do not convincingly support evidence of a direct effect. Such findings have repeatedly been shown to be consistent with the proposal to classify TPP as a suspected human reproductive toxicant CLP Category 2.
Additionally, the effects on male reproduction parameters are considered to be secondary to systemic toxicity due to the inconsistent nature of the effects, and several examples highlighting this are exemplified below:

1) A careful review of the data from this 2-generation study shows clear differences in the response of F0 and F1 males to treatment. The effect of 75 mg/kg/day PDDP on testes weights shows a discrepancy for the right organ (a slight effect in the F0 males, no effect in the F1 males) and the left testes (no effect in the F0 group, yet a significant effect in the F1 group). A direct acting reproduction toxicant would be expected to produce consistent effects between paired organs in treatment groups, and between generations. The inconsistency in effects within the same study suggest that the effects on the male reproduction system is indirect and occurs secondary to systemic toxicity.
2) Whereas there was a reduction in absolute mean left epididymides in the F0 males within the 2-generation study, there was no reduction in the F1 males who were treated much longer at 75 mg/kg/day. In fact, at 15 mg/kg/day mean weights were actually significantly higher. Again, this finding demonstrates clear inconsistencies in the observed effect of treatment within the same study.
3) In the 1-generation study oral gavage study, testes weights were reduced at 125 mg/kg/day contrary to findings in the 90-day dietary study where testes weights were increased at 100 and 150 mg/kg/day demonstrating an inconsistent effect among studies. It is our opinion that the absence of a consistent effect supports the conclusion that the observed effects on reproduction represent a secondary response to generalised toxicity. This lack of consistency in findings makes the quality of the evidence less convincing.

The UK Competent Authority suggests that the results of the 2-generation dietary study results are insufficient to overcome the findings of the 1-generation oral bolus gavage study. However, upon the completion and thorough analysis of this 1-generation study several years ago it was concluded that there were several potential confounding factors associated with the route of administration, the severity of systemic toxicity, and inconsistency of effects with earlier studies that could be clarified by the conduct of a definitive 2-generation dietary study preceded by a 90-day systemic toxicity range-finding study. The outcomes of the 2-generation dietary reproduction study are considered more reliable because 1) it avoids the recognised confounding influence of toxicokinetic considerations associated with the oral bolus gavage overwhelming the hepatic

detoxification system, 2) in addition to a desire to avoid the severity of generalised toxicity observed at 125 mg/kg/day in the 1-generation study the dosing regimen chosen for the 2-generation study evaluated a greater number of doses below the threshold for systemic toxicity with the specific intention of identifying evidence of direct effects of the substance on reproduction that were not secondary to systemic toxicity. In fact the dosing regimen chosen for the 2-generation study was based on the findings of the 90-day dietary systemic toxicity study, and took into account the known systemic toxicity profile of the substance, and dose levels were selected following the advice of an independent consultation with a recognised expertise in reproduction toxicology (Dr. Rochelle Tyl of the Research Triangle Park Institute, North Carolina USA), 3) it provided a more robust assessment of TPP effects over multiple generations (i.e. parental, F1 and F2 generations) including longer durations of treatment (conception through adulthood) in the F1 generation, 4) it assessed the effects of TPP over a greater variety of reproduction endpoints, and 5) the 2-generation dietary study is regarded by most experts as a more definitive assessment of reproduction toxicity potential compared with other test protocols.

RAC's response

Thank you for the comment. In the opinion of RAC, classification as Repr. 1B, H360F, is warranted.

RAC is of the opinion that the small reduction of litter size and of fetal body weight and single malformations occurring in 1-3 fetuses of 1-2 litters in the group of 23 litters of dams exposed to TPP at a dose of 300 mg/kg /day are due to significant maternal toxicity. No developmental toxicity was seen in foetuses in the groups exposed at 20 and 100 mg/kg/day. RAC notes that the TPP at a dose of 500mg/kg/day induced high maternal lethality and at dose of 300 mg/kg/day induced significant maternal toxicity leading to considerable reduction of the body weight gain during pregnancy (by ca. 30%; from 153g in control group to 107g in the 300 mg/kg group).

Therefore classification for developmental toxicity was not supported by RAC. The malformations were considered to be more likely to be spontaneous rather than treatment-related since they occurred in single foetuses.

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	Belgium		MemberState	19

Comment received

We have doubts regarding the classification proposal for reprotoxicity.
In the Edwards's study (2010), the toxicity on male reproductive parameters is observed at 75mg/kgbw : decrease of fertility indices and sperm concentration, the decrease of epididymides, prostate, seminal vesicles, testes weight,…, At this dose level, systemic toxicity is observed : decrease body weight, renal mineralisation,… In the Harriman's study (2004), systemic toxicity and toxicity on male reproductive parameters are observed at the same dose level as well. Indeed, it is reported that decrease body weight, small prostate, epididimide and vesicles seminale, soft faeces, hair loss, perianal staining. However, those systemic effects are not considered as market systemic toxicity as no lethality, dramatic reduction in absolute body weight, coma have been reported. As we consider the systemic effect not severe enough to establish a relationship with the fertility toxicity observed, we don't support the fact that fertility toxicity is considered as secondary to the systemic toxicity. Besides, no mating behavior , explaining parental effects instead of reproduction toxicity,has been observed.
We would therefore suggest the classification Repro Cat.1B H360F (according CLP criteria),

Cat.2 R60 (according to DSD criteria).
Dossier Submitter's Response
We thank the Belgian Competent Authority for their useful comments. We agree that long-term treatment of rats with TPP resulted in effects on reproduction parameters in a dose-responsive fashion but, as the following demonstrates, the entire cohort of data does show a demonstrable, close relationship between the effects on reproduction and concomitant systemic toxicity. In a manner similar to the response given to the French Authority, we offer a more comprehensive explanation: A collated review of the set of repeated-dose studies available for TPP illustrates the potential of this substance to induce a spectrum of generalised systemic toxicity: across all repeated-dose studies, effects observed on reproduction parameters were always associated with evidence of generalised systemic toxicity. This is exemplified by a consistent differentiation between doses that result in concomitant systemic toxicity and effects on reproduction parameters and those doses that do not result in systemic toxicity or reproduction effects (please refer to shaded dose-observation relationships illustrated in Tables 26 through 45 of the CLH dossier). This observation is further supported by the results from the 90-day dietary study where systemic toxicity resulted in significant reductions in body weight (absolute and gain) which pre-emerged at 50 mg/kg/day before any effect on reproduction parameters was observed. Adverse effects on reproduction parameters were only evident at the next higher dose (100 mg/kg/day) It is commonly recognised that when repeated dosing of a substance causes generalised systemic toxicity which precedes a secondary effect on an organ or tissue, the cessation of treatment results in a reversal of that secondary effect. This is exactly what was observed in the 28-day gavage study with high dose recovery groups (Harriman, 2003). In this study, at the end of the 14-day treatment recovery period systemic toxicity induced by TPP dissipates followed by evidence of a similar recovery among reproduction parameters as well. This series of observations support the conclusion that the effects of TPP on reproduction parameters occur secondary to generalised systemic toxicity, and is thus consistent with a proposal to classify the substance as a suspected human reproductive toxicant CLP Category 2. The Belgian Competent Authority makes the observation that all repeated-dose studies show some effect on reproductive endpoints in the male. It must be noted, however, that with only one or two minor exceptions none of these effects were evident in the absence of systemic toxicity. This conclusion is supported in the public consultation comments submitted by Competent Authorities from the Netherlands and Sweden, as well as industry responses from Chevron Oronite SAS, the submitter of the second dossier proposing a different classification. Additionally, the conclusion that the *effects on male reproduction parameters are secondary to systemic toxicity is exemplified by the lack of consistency in effects observed.* Several examples are brought forward below: 1) A careful review of the data from the 2-generation study shows clear differences in the response of F0 and F1 males to treatment. The effect of 75 mg/kg/day TPP on testes weights shows a discrepancy for the right organ (a slight effect in the F0 males, no effect in the F1 males) and the left testes (no effect in the F0 group, yet a significant effect in the F1 group). A direct acting reproduction toxicant would be expected to produce consistent effects between paired organs in treatment groups, and between generations. The inconsistency in effects within the same study suggest that the effects on the male reproduction system is indirect and occurs secondary to systemic toxicity. 2) Whereas there was a reduction in absolute mean left epididymides in the F0 males within the 2-generation study, there was no reduction in the F1 males who were treated much longer at 75 mg/kg/day. In fact, at 15 mg/kg/day mean weights were

actually significantly higher. Again this finding demonstrates clear inconsistencies in the observed effect of treatment within the same study.

3) In the 1-generation study oral gavage study, testes weights were reduced at 125 mg/kg/day contrary to findings in the 90-day dietary study where testes weights were increased at 100 and 150 mg/kg/day demonstrating an inconsistent effect among studies. It is our opinion that the absence of a consistent effect supports the conclusion that the observed effects on male reproductive organs represent a secondary response to generalised toxicity. This lack of consistency in findings makes the quality of the evidence less convincing

RAC's response

Thank you for the comment. In the opinion of RAC, classification as Repr. 1B, H360F, is warranted.

Date	Country	Organisation	Type of Organisation	Comment number
30.01.2013	Netherlands		MemberState	20

Comment received

Effects on sexual function and fertility

The dossier submitter proposes classification for effects on sexual function and fertility in category 2 because:

1. There were substance induced effects on sexual function and fertility.
2. However, these effects were only observed at dose levels also inducing systemic toxicity
3. The lack of reproducibility of the effect in dogs calls into question the relevance of the effect in rats to humans.

We agree with (1) that the available data show clear effects of phenol dodecyl branched on sexual function and fertility.

We also agree that these effects were only observed at dose levels also inducing systemic toxicity. However, the criteria state that it should be considered whether the effects on sexual function and fertility are a secondary non-specific consequence of other toxic effects. This has not been shown. The main other effect is reduced body weight and body weight gain. As discussed below the effects on sexual function and fertility are unlikely to be a secondary non-specific effect of the reduced body weights.

Correlation body weight and reproductive organ weight (males)
Decreases in the weight of male reproductive organs were often attributed to a decreased body weight gain. However, a large difference in reduction between terminal weight and reproductive organ weights was observed in the 14-d rat study, 28-d gavage rat study and both 90-d rat studies. The 14-d and 28-d repeated dose studies showed a reduction in terminal body weight of up to 13%, while the weights of seminal vesicles, prostate and epididymides at the same dose group were reduced with at least 50% as compared to control animals. The 90-d rat study from Haas (2007) revealed a reduction in male body weight of 35% in the highest dose group, while the prostate weight and seminal vesicle weight were reduced with 78-83%. This effect was also observed in the one-generation study, where body weight was reduced with 28% in males and the seminal vesicle weigth and prostate weight were reduced with 44 and 36% respectively.

Several publications have examined the relationship between body weight changes on organ weight data 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 as 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 lower 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 0-20% and at 40% body weight reduction, prostate and seminal vesicle weights were reduced 20-45%.

These data show that the reduction in reproductive organ weights cannot be fully attributed to reductions in body weight. However, also some other toxic effects were observed in males at dose levels also inducing effects on the male reproductive organs. This includes an increase in adrenal weight. As the adrenal has several functions it is unclear whether the change is related to changes in sexual hormones or other hormone systems. Further, some clinical effects were observed. However, they are unlikely to be the cause of the effects on sexual function and fertility. Overall the available data suggests that specific toxicity to the reproductive system cannot be excluded.

Effects in females
Four studies included an evaluation of effects in females. A 28-d study showed a 24-28% reduction in ovary weight at the two highest dose group, accompanied by decreased numbers of corpora lutea. At these dose levels, the body weight was not changed as compared to the control group and only clinical signs (excessive salivation and perianal staining) were indicative of any systemic toxicity. Further, the ovary weight reduction of 40% in a 90-d study is much higher than the body weight reduction of 18%, both observed in the highest dose group. Publications (as described above) have shown that a 15% body weight reduction correlates to an ovary weight reduction of 0-20%, whilst at 40% body weight reduction ovary weight is reduced by 13%. This indicates that the effect on ovary weight cannot be fully explained by changes in body weight.
In addition to these findings, the one-generation study showed a reduction in ovary weight of up to 30% at 125 mg/kg bw/d and an increased number of females with a persistent oestrus and dioestrus. The incidence of cysts in the ovary and endometrial gland were increased and the number of corpora lutea was decreased at 125 mg/kg bw/d. Similar effects on ovary weight, oestrus and corpora lutea were observed at 75 mg/kg bw/d in the F0 and F1 parental animals of the two-generation dietary study and cannot be fully explained by changes in body weight.

Again, the provided data indicates that the effects on the female reproductive organs cannot be fully explained by the changes in body weight. However, there are some other toxic effects such as effects on the adrenals which cannot be excluded as primary cause.

There is no mechanistic information on phenol, dodecyl branched that could further clarify whether the effects on sexual function and fertility are a secondary non-specific consequence of the other toxicity. However, some information is available for nonylphenol (branched). There is a strong structural relation between nonylphenol and phenol, dodecyl branched as both substances consist a phenol group with an alkyl tail. Further, nonylphenol shows comparable effects on sexual function and fertility. This justifies read-across.

Reproduction toxicity nonylphenol
The observed effects are indicative of an oestrogenic effect of phenol, dodecyl, branched. This is consistent with the conclusions for nonylphenol as reported in 2002 in the EU Risk

Assessment Report (available at: http://echa.europa.eu/web/guest/information-on-chemicals/information-from-existing-substances-regulation?search_criteria=phenol). In this report it is concluded that nonylphenol has oestrogenic activity (potency 3 to 6 orders of magnitude less than that of oestradiol) and that nonylphenol exposure over several generations can cause minor perturbations in the reproductive system of offspring, namely slight changes in the oestrous cycle length, the timing of vaginal opening and possibly also in ovarian weight and sperm/spermatid count, although functional changes in reproduction were not induced at the dose levels tested. The observed perturbations in offspring are compatible with the predictable or hypothesised effects of exogenous oestrogenic activity. Evidence of testicular toxicity, seen as seminiferous tubule vacuolation, cell necrosis and a reduction in tubule diameter, was reported at exposure levels which also cause mortality in a repeated dose gavage study in rats.
Based on the data available in the RAR, nonylphenol has a harmonized classification as toxic to the reproduction in category 2 (CLP) for both effects on development and of sexual function and fertility.
In addition, nonylphenol and substances containing nonylphenol have been designated as an endocrine disruptor (http://echa.europa.eu/documents/10162/baa009d8-5d13-4fcc-bf6a-c68de5aade10) also indicating that nonylphenol has specific effects on hormones in mammals. After publication of the RAR several additional studies have been published Further, several studies regarding effects on sexual function and fertility of nonylphenol been published including amongst others:

C. De Jager, M. S. Bornman, G. van der Horst I. The effect of p-nonylphenol, an environmental toxicant with oestrogenic properties, on fertility potential in adult male rats. Andrologia Volume 31, Issue 2, pages 99–106, March 1999

Vendula Kyselova, Jana Peknicova, Daniela Buckiova and Michael Boubelik
Effects of p-nonylphenol and resveratrol on body and organ weight and in vivo fertility of outbred CD-1 mice. Reproductive Biology and Endocrinology 2003, 1:30

In our opinion the results of these and onther studies with nonylphenol are relevant for the classification of phenol, dodecyl branched.

Overall, there is evidence suggesting that nonylphenol has specific effects on the hormonal system resulting in adverse effects on the sexual function and fertility. Seen the comparable structure and effects, it is considered likely that phenol, dodecyl branched also has specific effects on sexual function and fertility.

The dossier submitter questions the relevance of the effects in rats for humans due to the absence of comparable effects in dogs (item 3). However, it is unclear from the available summary of that study whether the young dogs used in that study already reached adulthood. Pre-pubertal dogs may be less sensitive to substances affecting the reproductive tract. Please provide information on age and sexual status (puberty or adults of the male dogs at necropsy). See:
M. A. TAHA, D. E. NOAKES, W. EDWARD ALLEN. Some aspects of reproductive function in the male Beagle at puberty. Journal of Small Animal Practice Volume 22, Issue 10, pages 663–667, October 1981)
However, even is the dog data show that the effect on sexual function does not occur in dogs, it remains unclear whether humans more resemble rats or dogs in this aspect. Seen the clear effects in several species of nonylphenol, it is considered likely that other alkylphenols including phenol, dodecyl branched also induces such effects in many species including humans.

Summarized, the adverse effects on reproductive organ weight in males and in organ weight and reproduction parameters in females cannot be fully explained by systemic toxicity. In addition, the effects are indicative of oestrogenic activity of phenol, dodecyl, branched, which is in line with the conclusions on nonylphenol.
According to the criteria for Category 1B (presumed reproductive toxicant) data shall provide clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or if occurring with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. The data indicate that the effects are not a secondary non-specific consequence of systemic toxicity and the findings are in line with a possible oestrogenic effect of phenol, dodecyl, branched. Category 1B classification (CLP) is therefore considered appropriate.

Dossier Submitter's Response

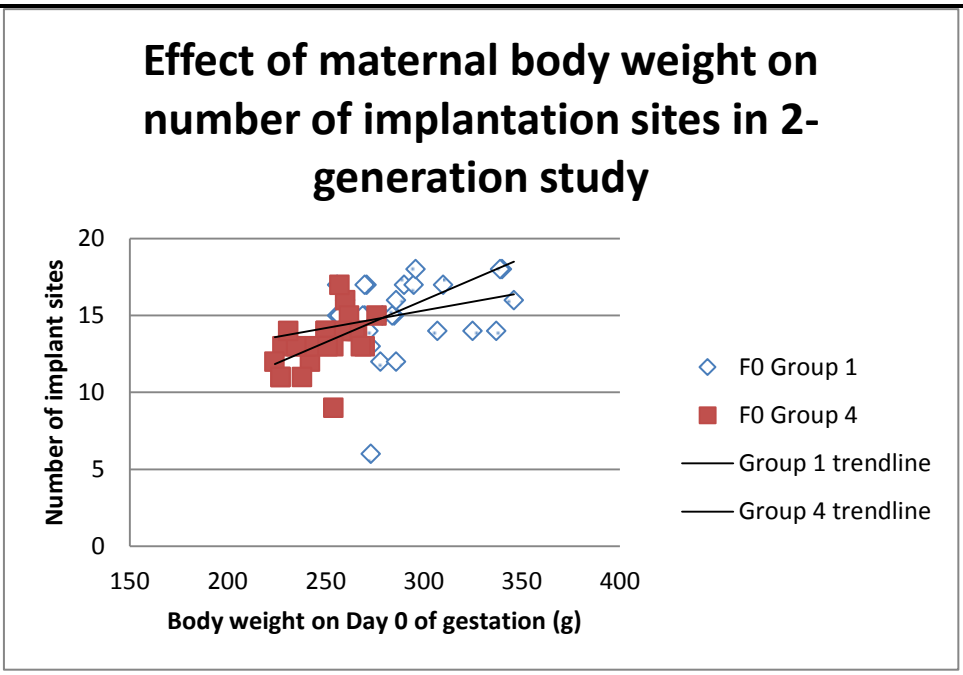
We thank the Dutch Competent Authority for their valuable comments. We agree with their comment that effects of TPP on reproduction are only observed at dose levels that induce systemic toxicity (please refer to shaded dose-observation relationships illustrated in Tables 26 through 45 of the CLH dossier). However, we do not agree with their conclusion that the effects of TPP on reproduction parameters do not occur secondary to generalised systemic toxicity for the following reasons:
The Competent Authority make the observation that all repeated-dose studies show some effect on reproductive endpoints in the male. It must be noted, however, that these effects predominantly occurred secondary to the presence of systemic toxicity. The conclusion that the effects on male reproduction parameters are secondary to systemic toxicity is further exemplified by the lack of consistency in effects observed. Several examples are brought forward in previous responses to the French and UK Competent Authorities.
In regard to the the Competent Authority's questioning the relationship between the effect of body weight reductions on reproductive endpoints it appears that there is a misinterpretation that the effects observed on reproduction were directly attributed only to reductions in body weight. In fact, indicators of systemic toxicity seen in the repeated-dose studies included haematological changes, adverse effects on organs including the liver, heart, kidney, clinical observations as well as effects on body weight / food consumption / efficiency of food utilisation. These changes were <u>all</u> indicative of the generalised systemic toxicity produced by the substance, of which effects on body weight / body weight gain was the most frequently observed indicator. Additionally, whilst it is accurate to suggest that food deprivation / dietary restriction leads to adverse body weight effects along with adverse effects on reproductive organ function, these findings are more plausibly due to a direct nutritional effect rather than an indicator of generalised systemic toxicity. In contrast, the reductions in bodyweight development observed in the studies with TPP are considered to be a sensitive indicator of and symptomatic of generalised systemic toxicity that is affecting the overall systems function on the whole animal. This conclusion is supported by the dose-response relationship of generalised systemic toxicity resulting from TPP treatment (please refer to the response to the French Competent Authority). Therefore it is not entirely suitable to directlycompare the extent of the bodyweight and accompanying reproductive changes seen in response to restriction of adequate nutrition described in the public literature on dietary restriction studies with the phenomenon that is observed in the repeated-dose studies using TPP.
The Competent Authority also cites the effects of TPP on ovarian weight, implantation site, corpora lutea, ovarian cysts, and estrus cycling in the 1- and 2-generation studies as justification for classification.
Concerning the effects on ovary weights, a review of the repeated-dose studies in rats indicates that TPP caused a reduction in ovary weights at 125 and 25 mg/kg/day in the 1-generation study, and at 75 mg/kg/day in the 2-generation study. However, it is relevant to mention that there were no corresponding effects on ovary weights at 50 mg/kg/day in the

90-day dietary study, nor in the 28-day gavage study at 60 mg/kg/day. These observations highlight the inconsistency of effects between studies. Together with unconvincing evidence of an associated effect on female fertility at dose levels not causing generalised toxicity (which has typically been considered necessary to justify classification of reprotoxic substances at a higher severity of hazard), the absence of consistent effects on ovaries between different studies supports the current proposal to classify TPP as a suspected human reproductive toxicant, Category 2.

The absence of consistent effects on ovaries is supported by public comments from Chevron Oronite SAS citing previously undisclosed 2-generation studies on high molecular weight substances containing relevant amounts of residual TPP. A multi-generation study showed that administration of TPP at an estimated 67 mg/kg/day had no significant effect on ovarian weight in the F1 females, and there were no adverse histological findings in the ovary at any dose or in any treated generation (Nemec et al 1995). Similarly, another 2-generation study on a different high molecular weight substance containing an estimated 38 mg/kg/day residual TPP was reported to have no significant effect on ovarian weight in any generation and no accompanying adverse histological findings on this reproduction organ (Wood et al 2002). Finally, a different 2-generation study is cited where an estimated 39 mg/kg/day residual TPP produced no significant effect on ovarian weight in any female generation and no accompanying adverse histological findings (Wood et al 2003). This set of results supports the conclusion that treatment at doses below the threshold level shown to elicit generalised systemic toxicity (namely 100 mg/kg/day TPP in the 90-day toxicity study; 75 mg/kg/day TPP in the 2-generation study) results in inconsistent and unreliable effects on ovarian parameters. For this reason we would argue that treatment-related effects on ovaries are unreliable indicators of a direct effect of the substance on reproductive parameters.

In terms of consistency of effects inconsistent effects on uterine weight, female rats treated with 125 mg/kg/day in the 1-generation reproduction toxicity study responded with no statistically significant change in uterine weight whereas female rats dosed at 75 mg/kg/day in the 2-generation dietary study were found to have a non-statistically significant reduction in uterine weight. These findings contrast to those of the uterotrophic assay which showed a significant increase in wet and blotted uterine weights over the dosing range from 75 to 500 mg/kg. These findings are themselves inconsistent with the observed reduction in absolute and relative uterus weights in the female pubertal assay over the range of doses 50 to 300 mg/kg/day, including uterine hypoplasia at 60 mg/kg/day and above. The inconsistency in the effect of TPP on uterine response over these multiple study designs makes the quality of the conclusion of a mechanism of action less convincing and raises doubt about the relevance of this finding to humans.

The Competent Authority also raises a cogent point related to the effect of TPP on implantation sites. In the definitive 2-generation study, a regression analysis of individual F0 animal data at 75 mg/kg/day shows a linear relationship between the reduction in body weight (indicative of secondary generalised systemic toxicity) and a reduction in implantation sites (see table below). The analysis supports the conclusion that this endpoint response is secondary consequence to systemic toxicity manifested by a reduction in body weight development.



F0 Group 1 = Control animals; F0 Group 4 = Animals treated with 75 mg/kg/day tetrapropenylphenol
The trendlines both show a positive correlation between body weight and the number of implantation sites; the "steeper" trendline shows how toxicity causes secondary weight loss and is correlated directly with the observed reduction in implantation sites.

The Competent Authority also notes the perturbations of estrus as a result of TPP. Measurement of estrus cyclicity is a highly imprecise endpoint with a high degree of variability and that a number of confounding factors can influence measurements (e.g., disturbances in estrous cycle can result from mishandling the animals). This is substantiated by the observation in the 2-generation assay that control animals in the F1 group had a 27% incidence of persistent diestrous. The technical imprecision of this endpoint combined with the degree of systemic toxicity observed at 75 mg/kg/day reduces the reliability of any conclusion about direct effects of TPP on reproduction.
The Competent Authority raises a valid and interesting analogy with other alkyl phenols that have been evaluated for effects on reproduction. In fact, there is a high degree of structural similarity between nonylphenol and this substance. The toxicological similarity between nonylphenol and TPP is established by reviewing data extracted from key endpoints published on the ECHA dissemination website. Nonylphenol has been observed in rat studies to cause effects on ovary weight, estrus cycling, changes in time to vaginal opening, effects on accessory glands and minor perturbations in the reproduction system of offspring. A comparison of the findings from reproduction toxicity studies shows that treatment of rats with nonylphenol over 3 generations resulted in a reproduction NOAEL level = 15 mg/kg/day. Similarly, TPP in a 2-generation test also yielded a NOAEL level = 15 mg/kg/day in the same species. There already exists a harmonised classification for nonylphenol as a suspected human reproductive toxicant, Category 2. Given the recognised high degree of similarity between nonylphenol (structural and toxicological) and TPP it is appropriate to presume that there will be a consistent approach to classification and this supports the current proposal to classify TPP as a suspected human reproductive toxicant, Category 2.
Finally in response to the question about the age and state of sexual maturity of the test animals in the 90-day dietary study in dogs we can confirm that the beagle dogs were 6-8 months old at the start of the study. A literature citation indicates sexual maturity in male beagles is established at 26-28 weeks of age (Kawakami, et al;, Histological observations of the reproductive organs of the male dog from birth to sexual maturity Journal of Veterinary Medical Science, Japanese Society of veterinary Science, 1991. Vol 53(2) pgs. 241-248.

RAC's response
Thank you for the comment and the analysis of the data. In the opinion of RAC, classification as Repr. 1B, H360F, is warranted. See also RAC response to comment No. 17.

Date	Country	Organisation	Type of Organisation	Comment number
29.01.2013	United Kingdom		Company-Downstream user	21
Comment received				
Whilst effects on fertility have been demonstrated in a one-generation study there is no clear evidence of specific reproductive toxicity in the absence of other toxic effects. Since no treatment related adverse effects on reproductive parameters could be determined at dose levels that did not elicit general systemic toxicity in both the one generation and definitive two generation study, we support the proposed classification as Category 2 Reproductive effects (adverse effects on sexual function and fertility.				
Dossier Submitter's Response				
We appreciate your support of the classification				
RAC's response				
The option of classification of TPP as Repr. 2 was considered by RAC; however, classification as Repr. 1B was considered justified based on the information provided to RAC. The rationale and justification for the classification as Repr. 1B is contained in the opinion document.				

Date	Country	Organisation	Type of Organisation	Comment number
23.01.2013	United Kingdom		Company-Importer	22
Comment received				
My company supports the proposed hazard classification as Category 2 for reproductive toxicity for the following reasons: • Upon critical review of the available data, the weight-of-evidence from the different studies included in the harmonization dossier demonstrates that the adverse effects on reproduction / fertility seen resulted primarily from non-specific systemic toxicity rather than a specific effect on fertility, reproductive organs and/or sexual function. • Non-specific systemic toxicity manifested itself in the rat model as severe morbidity and mortality at high doses, adverse clinical observations at moderate doses, and significant reductions in body weight / body weight gain at lower doses. There were no toxicologically-significant findings on reproduction / developmental parameters in the absence of adverse effects on bodyweight. It is relevant to mention in this context that when recovery group animals were included in the study design, adverse findings on reproductive parameters were shown to be reversible in parallel with the reversal of systemic toxicity (pages 43-44 and 61-64). • It is significant that the marked effect on fertility observed in the one-generation reproductive toxicity study (pages 56-57 and 68-73) was not observed at similar dose levels in a two-generation study (pages 56-57 and 74-85) using a different method of test material administration (i.e. bolus administration by gavage once each day for the one-generation study vs. continuous administration throughout the day in the diet for the two-generation study). The absence of consistent effects between the two studies on markers of fertility and sexual function is considered to be an important differentiator for category of classification. For example, gavage administration of Phenol, dodecyl-, branched in the one-				

generation study resulted in adverse effects on sperm parameters whereas these effects were not observed in the two-generation study utilising dietary administration. The marked difference in toxicologically-significant findings between the two studies strongly suggests that the nature and extent of the changes observed in the one-generation study are more likely to be due to a bolus effect of gavage administration that results in greater systemic toxicity as opposed to a direct effect on the rodent reproductive tract. For such findings to be considered relevant to humans, one would expect to see similar adverse effects in the two-generation study where a method of administration was more relevant to the temporal pattern of possible human exposure.

• In addition to a lack of consistency of effects on fertility and reproduction cited above, there is also an inconsistent effect on these parameters between different species. For example, adverse effects on the male reproductive tract in the rat were not evident in the dog following 90-day administration of this substance (pages 47 and 68).

• Support for the current classification proposal can be found in previous RAC opinions concerning the classification of other substances for reproductive toxicity (pages 90-92). Specifically, the RAC has included one or more of the following as justification of their decision to assign a substance to a specific reproductive hazard category:

a. Effects on the male reproductive tract are relevant for general classification as a reproductive hazard. However in previous opinions, the RAC has considered that there should be accompanying evidence of adverse consequences for male fertility in order to justify Category 1B. Effects were seen on the rodent male reproductive tract following administration of Phenol, dodecyl-, branched but were without consequence for fertility except in one study where there was concomitant systemic toxicity associated with bolus gavage administration at high doses of test substance. Significantly, there were no toxicologically-significant effects on fertility in a two-generation study following continuous (non-bolus) administration of Phenol, dodecyl-, branched in the diet.

b. The RAC cites, in previous opinions, the lack of consistency of effects in different studies within the same species, following different routes of administration, or between different species, as justification for a Category 2 classification for reproduction. The studies described in the dossier do not demonstrate a consistent effect of Phenol, dodecyl-, branched on reproductive tissues and fertility either within the same species or between species.

c. The significance of the method of administration has been previously recognised by RAC as a potential confounding factor in any assessment of treatment-related findings. For example, we note that Lucirin was assigned to Category 2 for reproductive toxicity because there were inconsistent effects observed between studies using different methods of administration; this inconsistency was considered to be attributable to a bolus effect of gavage dosing.

d. The association of the reproductive findings with general toxicity are commonly taken into consideration by RAC. Substances causing reproduction effects associated with systemic toxicity are typically assigned to a category of lower concern than when effects are seen in the absence of non-specific toxicity.

Dossier Submitter's Response

We appreciate the additional information provided and your support of the classification

RAC's response

The option of classification of TPP as Repr. 2 was considered by RAC; however, classification as Repr. 1B was considered justified based on the information provided to RAC. The rationale and justification for the classification as Repr. 1B is contained in the opinion document.

Date	Country	Organisation	Type of Organisation	Comment number

18.01.2013	Germany		Company-Manufacturer	23
Comment received				
Repro: data from a valid 2-generation study according to OECD 416 are available. The data demonstrate that the substance is not toxic to reproduction, i. e. does not impact the fertility or development of offspring at doses that are not toxic to parental animals. The effects observed occur only at doses that also result in systemic toxicity of the maternal animals (like bodyweight effects, organ weight effects etc.). We therefore support the proposed classification as Cat. 2, Reproductive effects (adverse effects on sexual function and fertility). The data from a valid developmental toxicity study justify the decision for "no classification" for "Reproductive toxicity" (adverse effects on development of the offspring), since effects were only observed in the presence of maternal toxicity with marked toxicity in individual dams.				
Dossier Submitter's Response				
Thank you for your response. We agree with the position that existing data supports the classification of the substance as Repr. Cat. 2 (CLP Regulation).				
RAC's response				
The option of classification of TPP as Repr. 2 was considered by RAC; however, classification as Repr. 1B was considered justified based on the information provided to RAC. The rationale and justification for the classification as Repr. 1B is contained in the opinion document. RAC is of the opinion that classification for developmental toxicity is not justified for the reasons also provided by you (also see RAC response to comment No 18).				

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Sweden		MemberState	24
Comment received				
Please see attachment. --- *ECHA's comment: The text below is copied from the attachment COM_CLH_PC_Phenol dodecyl branched_SE.docx* **Reproductive toxicity** : The Swedish CA supports the proposed classification of Phenol, dodecyl-, branched / Phenol, (tetrapropenyl) derivatives (CAS nr 121158-58-5, 74499-35-7) as a reproductive toxicant in category 2 regarding effects on fertility. In addition we propose that it also should be classified for developmental toxicity (category 2) and for effects via lactation. <u>Developmental toxicity</u> In an OECD guideline 414 study, two fetus (from different litters) with ectrodactyly were found in the high dose group. One of these fetuses also had brachydactyly and absent claw. In addition a low incidence of malformations affecting the humerus, ulna, radius or femur were also recorded and a number of fetuses (10) displayed the malformation "scapula/scapular spine curved" at the highest dose level (300 mg/kg). Data is lacking that clarifies the number of fetuses that were affected by one or more of these malformations. In addition, decreased litter size (8.9 as compared to 12.5 in controls) and a decreased fetal weigh (3.47 as compared to 3.76 g in controls) were also recorded at the high dose level. No similar findings were recorded in the lower dose groups and no historical control data is available.				

Maternal toxicity was observed at the 300 mg/kg dose level as decreased bodyweight gain Day 6-15 of (62 % lower than the gain observed for the controls) and decreased food intake (- 18% as compared to the controls). No mortality was recorded at this dose level and the only clinical sign that was recorded was soft stool. Animals at the 300 mg/kg dose level seemed to recoup somewhat after end of dosing, since the decrease in body weight gain was not as dramatic as the one observed during the dosing period (-16% as compared to the controls but most likely the decreased body weight gain is overestimated since the data has not been adjusted for the lower gravid uterus weights that was caused by the resorptions). The maternal toxicity observed at the high dose level can therefore explain the effects on fetal weights and probably also to some degree the increased number of resorptions. However there is no data in the present study that suggest a mechanism that link the observed maternal toxicity to the occurrence of the malformations, and we are not aware of any literature that link the general maternal toxicity as described in this dossier to the occurrence of specific malformations. No evidence of teratogenicity was recorded in the one and two generation studies that were performed at lower dose levels (high dose levels were 125 and 75 mg/kg, respectively).

In summary, we propose that Phenol, dodecyl-, branched / Phenol, (tetrapropenyl) derivatives should be classified as a category 2 reproductive toxicant regarding developmental toxicity. The "signal strength" in the data does not justify a classification in category 1b.

<u>Effects on fertility</u>
Signs of effects on the estrous cycling (increased numbers of females showing persistent diestrus/estrus) were seen in females dosed with 125 mg /kg in the one generation reproductive gavage rat toxicity study. A reduced fertility index was also observed and the mean number of implantations was radically decreased (3.3 as compared to 14.1 in the control group) and consequently the litter size was also much lower as compared to the control litter size. Histopathological examination revealed an increased incidence of females with a decrease in the numbers of corpora lutea and an increased incidence of females having ovary cysts and/or endometrial cysts in high dose females. The weights of the ovaries (absolute and relative to body weight or brain) were all statistically significantly decreased in the high dose group. No overt maternal toxicity was evident at time of pairing. During the pre-mating period the high dose females had a lower body weight gain (-21.3 % as compared to the controls) and at time just before mating the mean weight of the high dose females was 9.7% less than the weight of the control females. Interestingly an increased incidence of females with persistent diestrus was also observed in the intermediate (25 mg/kg) dose group (four animals as compared to 16 in the high dose and two in the control). No effects on fertility index or on litter size were observed for this dose group, but again a decrease in the weight of the ovary (absolute and relative to brain) was recorded. At this dose level the pre-mating body weight gain and weight just before mating were similar for the intermediate dose females and the control females.

Although no effects on fertility index or on litter size were noted in the females in the two generation dietary reproductive toxicity study, partly similar signs as those observed in the one generation study were observed. The weight of the ovary was reduced (75 mg/kg, F_0 and F_1) and an increased incidence of females with reduced number of corpora lutea (75 mg/kg, F_0 and F_1 females) and of females with ovary cysts (6/30, 13/30 and 10/30 in the control, 15 mg/kg and 75 mg/kg dose group, respectively in the F_0 generation) were observed. Vaginal patency was recorded in F1 females at an earlier time point (and at a significantly lower mean bodyweight) as compared to the controls. However no similar effect was noted in the one generation study and no effect on the anogenital distance was

seen in the F_2 generation. Maternal toxicity was recorded in this study, just before the mating period 75 mg/kg F_0 females weighed 12.6% less than the control females, and on lactation day 21 the difference in bodyweight was ~5% but no effects were observed for the 15 mg/kg F_0 females. The 75 mg/kg F_1 weighed 12.5% less than controls around mating whereas 15 mg/kg F_1 females weighed about the same as the controls around mating.

Interestingly, Chapin (Fundamental and Applied Toxicology 20, 23-29, 1993) reported for Sprague Dawley rats that feed restrictions resulting in a weight of 70% of the controls had no effect on fertility. Decreased ovary weights and decreased number of corpora lutea as well as a transient prolongation of the estrous cycle time were seen in female rats that weighed 70% of controls but not in rats that weighed 80 or 90%, i.e. a similar effect on bodyweights as the maximum effects recorded in the present studies.

Table 1. Summary of statistically significant effects of Phenol, dodecyl-, branched on male organ weights

		One – generation study (gavage)		Two - generation study (dietary)		
Organ	**Weight**	**Dose level (mg/kg)**		**Dose level (mg/kg)**		
		125 (F_0)	**25 (F_0)**	**75 (F_0)**	**75 (F_1)**	**15 (F_1)**
Pre-mating body weight	**Absolute (% of control)**	**79,4 %**	**96 %**	**82,7 %**	**77,5 %**	**95,1 %**
Adrenals	**Absolute**	↑				
	(%)	↑		↑	↑	
	(g/100g brain)					
Cauda epididymis	**Absolute**	↓	↓	↓	↓	↑
	(%)				↑	↑
	(g/100g brain)	↓	↓	↓		↑
Epididymis	**Absolute**	↓		↓	↓	↑
	(%)	↑			↑	↑
	(g/100g brain)	↓		↓		↑
Prostate	**Absolute**	↓		↓	↓	
	(%)				↑	
	(g/100g brain)	↓		↓		
Seminal vesicle	**Absolute**	↓	↓	↓	↓	
	(%)	↓		↓	↑	
	(g/100g brain)	↓	↓	↓	↓	
Testis	**Absolute**	↓		↓	↓	
	(%)	↑	↑	↑	↑	
	(g/100g brain)	↓				

As summarized in Table 1, Phenol, dodecyl- branched had an effect on male reproductive

organ weights. Effects were observed both in the one- and two-generation reproductive studies. Interestingly effects were also seen at dose levels that caused mild toxicity (as revealed by effects on pre-mating body weights and adrenal weight). Histopathological examination of the reproductive organs revealed no effects in the two- generation study, but in the one-generation study a reduced prostate secretion was observed in all dose groups, a reduced coagulation gland was found in males dosed ≥25 mg/kg and reduced seminal vesicle secretion was observed only at the 125 mg/kg dose group. Sperm analysis did not reveal any effects in males in any of the studies.

In summary, effects on male and female reproductive organ weights were observed in the one- and two-generation reproductive toxicity studies. Effects were generally observed at dose levels that caused some toxicity (as revealed by effects on the absolute body weight but no mortalities) but signs of similar effects were also observed at lower dose levels. Estrus cycling was affected in both studies as revealed as a somewhat prolongation of the estrus cycle time or as an increase in the number of females displaying persistent or prolonged diestrus or abnormal estrus. These effects were most pronounced in the high dose groups but effects were also recorded at lower dose levels. Decreased number of corpora lutea (high dose groups, $F_0$and F_1) and an increase in the no of females with ovary cysts were also observed. Overall no effect on sperm function was recorded. A significant decreased fertility was also recorded in the one generation study at the high dose level in combination with clear toxicity (but no mortalities). These effects indicate that the test compound can perturb the endocrine system and that at high dose levels this perturbation will affect fertility. A classification of phenol, dodecyl-, branched as a category 2 reproductive toxicant regarding effects on male and female fertility is therefore warranted.

Effects on or via lactation

In the two generation dietary study, five F_1 (sex distribution not specified in the report) animals in the high dose group (75mg/kg) were found dead or killed just after weaning due to their poor condition. Death occurred in animals with low body weight. In addition, an effect on pup weight was also a general phenomenon in this study and it was most pronounced in the F_1 generation. On day 1 the mean weights of the F_1 high dose pups were similar to the controls (96% [males] and 97% [females]) but on day 21, males weighed 28% less and females 30% less than the controls. During lactation maternal toxicity (as revealed by effects on maternal body weight) was minimal. The weight of the phenol, dodecyl treated dams at end of lactation (day 21) was only minimally lower (5.2%) as compared to the control group and the treated high dose dams actually gained more in weight during lactation (43 g) as compared to the control dams (25 g). In addition the influence of the mild maternal toxicity (the high dose dams weighed ~10% less on gestation day 20) that was observed during pregnancy at this dose level seemed to have had a negligible effect on the pups since there was no effect on pup survival or pup weight on day 1. In summary, the observed detrimental effect on pup growth justifies a classification for effects via lactation and thus the addition of H362.

Dossier Submitter's Response

We thank the Swedish Competent Authority for their comprehensive assessment of the harmonisation dossier, and we are in agreement with the conclusion agreeing with the proposed classification of this substance as a Category 2 reproductive toxicant for effects on male and female fertility. The Competent Authority points out that effects on reproduction parameters in the 1-generation oral gavage study, and to a lesser extent in the 2-generation dietary study, occur at doses which cause clear systemic toxicity, and that there were no convincing effects on fertility in the absence of clear toxicity. These findings have been addressed elsewhere in the context of comments made by other Member States. In addition, the Competent Authority recognises the inconsistency of effects on certain reproduction endpoints in the repeated dose studies, specifically vaginal patency and

anogenital distance. Whereas as an effect on vaginal patency in the F1 offspring was observed in the 2-generation dietary study, there was no such effect on vaginal patency in the F1 female offspring in the 1-generation reproduction oral bolus gavage study up to the high dose (125 mg/kg/day), and no effect on anogenital distance in the F2 offspring. The fact that no effect on vaginal patency was observed at a higher dose and by a different method of administration in a different study highlights the inconsistency in findings between studies. This makes the observation of an effect on vaginal patency in the 2-generation study less convincing, and thus lessens the potential relevance to humans. Furthermore, measurement of anogenital distance in F2 offspring after 2 full generations of TPP treatment showed no difference across all groups. Anogenital distance is an endpoint in rodents that is very sensitive to hormonal effects of test chemicals. Substances reported to cause anogenital distance alternations include those with androgenic, anti-androgenic and estrogenic properties. If TPP was causing <u>direct</u> adverse effects on reproduction by anti-androgenic or estrogenic mechanisms, one would expect consistent effects for similarly affected target parameters (preputial separation, vaginal patency, and anogenital distance) within studies as well as between the 2-generation and 1-generation studies. In fact this is demonstrably not the case for TPP which makes the argumentation for direct effects less convincing, and the relevance to humans more questionable.

<u>Developmental effects</u>
In the rat developmental toxicity study, increased incidences of external and skeletal malformations and variations were observed at the highest dose level of 300 mg/kg bw/d. Marked maternal toxicity was observed in this group and there is a clear association between litters with malformations and individual dams with the most marked bodyweight effects in this study. It is also notable that an additional group of rats administered 500 mg/kg bw/d was terminated early due to excessive mortality. The effects seen at 300 mg/kg bw/d therefore have been considered to be secondary to maternal toxicity and not to represent a direct toxic effect. Given the signal strengths of the external variations and malformations observed at 300 mg/kg, we agree that the study results deserve further discussion on whether those abnormalities meet the criteria for classification for developmental effects as Category 2.

<u>Lactation effects</u>
Although the 2-generation study used dietary concentrations adjusted to give constant intakes of the test material, dietary concentrations were not adjusted during the lactation period. As a consequence of the much higher food consumption by maternal rats, this meant that administration of the test material during the lactation period was markedly above the nominal dose levels. In fact, test material intakes attained during the lactation period at the nominal dose level of 75 mg/kg bw/d were estimated to be 174 (115-205), 166 (105-187) and 158 (102-188) mg/kg bw/d for the F1, F2A and F2B litters, respectively. These dose levels can be predicted to be associated with marked maternal toxicity and therefore the effects on post-natal survival are considered more likely to be secondary to generalised maternal toxicity instead of representing a direct toxic effect. Nevertheless we agree that this endpoint requires further evaluation and discussion.

RAC's response

Thank you for your comment.

The option of classification of TPP as Repr. 2 was considered by RAC; however, classification as Repr. 1B was considered justified based on the information provided to RAC. The rationale and justification for the classification as Repr. 1B is contained in the opinion document.

RAC is of the opinion that the small reduction of litter size and of fetal body weight and

single malformations occurring in 1-3 fetuses of 1-2 litters in the group of 23 litters of dams exposed to TPP at a dose of 300 mg/kg /day are due to significant maternal toxicity. No developmental toxicity was seen in foetuses in the groups exposed at 20 and 100 mg/kg/day. RAC notes that the TPP at a dose of 500mg/kg/day induced high maternal lethality and at dose of 300 mg/kg/day induced significant maternal toxicity leading to considerable reduction of the body weight gain during pregnancy (by ca. 30%; from 153g in control group to 107g in the 300 mg/kg group).

Therefore classification for developmental toxicity was not supported by RAC. The malformations were considered to be more likely to be spontaneous rather than treatment-related since they occurred in single foetuses.

Classification for effects on or via lactation was not supported by RAC since the observed effects do not meet the CLP classification criteria for this category.

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	France	Chevron Oronite SAS	Company-Manufacturer	25

Comment received

Comments to: CLH Report Proposal for Harmonized Classification and Labeling
Substance Name: Phenol, dodecyl-, branched (abbreviated TPP)
EC Number: 310-154-3
CAS Number: 121158-58-5
Dossier Submitter: SI Group-UK, Ltd
Version number: 4
Date: 10th October 2012

Dear ECHA:
Thank you for the opportunity to comment upon the aforementioned dossier. We present to you the following comments for review:
o Data derived from TPP-containing substances support a proposed SCL of 1.5% for reproductive toxicity.
o We recommend that a SCL of 1.5% be adopted for TPP. A SCL of 1.5% would be based upon adequate, reliable and conclusive scientific information and would classify TPP-containing substances that pose TPP's reproductive hazards, and would not place unnecessary restrictions upon TPP-containing substances that do not present this potential hazard. COSAS currently self-classifies TPP as Repr. 1B and applies the 1.5% SCL globally.
o The 1.5% recommended SCL is validated by the reproductive toxicity test data of four TPP-derived substances containing 2.5% to 26% TPP. These studies were conducted for substance registrations prior to REACH
o Four mechanistic studies (two in vivo and two in vitro) are also discussed, which support our earlier reproductive toxicity findings.
o We concur with the opinion stated in the SIG submission that effects observed upon male reproductive organs may be secondary to systemic health effects. Additional support for the submitter's position is described in the accompanying pages.
o The CLH report was submitted prior to issuance of the final report for the two-generation (Edwards, 2012) reproductive toxicity study, and does not include the interpretation by the independent laboratory that conducted this study.
o The final report is not included in the summary of data available at the ECHA website [http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances]
o Female reproductive toxicity does not appear to be entirely attributable or secondary to

systemic health effects.

We welcome the opportunity to answer questions in response to these comments and appreciate the opportunity to participate in the public commentary.

Comments to the CLH Proposal for TPP

1. **Data from TPP-containing substances support a SCL of 1.5% for reproductive toxicity for TPP-containing substances.** We recommend that the SCL for TPP be adopted at 1.5%, equivalent to 15 mg/kg/day. Based upon a full examination of the toxicity of TPP-containing substances, the most sensitive, consistent change to the reproductive system observed from TPP administration was a reduction in ovary weight. This alteration occurred in a dose-responsive manner to exposures as low as 25 mg/kg/day, but did not occur at 15 mg/kg/day in either generation of the two-generation (Edwards, 2012) reproduction study.

a. We have also calculated the SCL using the ED10 approach as recommended by ECHA guidance [Guidance on the Application of the CLP Criteria. Version 3, Nov. 2012]. ED10 analyses were used to evaluate dose-responsive data generated in the two-generation (Edwards, 2012) and one-generation (Knapp, 2006) studies. The ED10 values and their associated lower limits for the 95% statistical confidence interval, ED10Low, inform about the point of departure for dose-responsive effects. The USEPA software BMDS (version 2.3.1) was used to estimate the ED10 and ED10Low values for ovary weight as the most sensitive reproductive endpoint in each study. The data for calculations differ in that the ovary/oviduct weights were measured in the Knapp 2006 study, whereas the ovary weights were measured without the oviducts in the Edwards, 2012 study. A benchmark response (BMR) of one standard deviation below the control mean value was selected, as this adequately represents the risk of approximately 10% of a population exhibiting a detectable change in a continuous endpoint (Crump 1995).

All of the continuous models in the BMDS software suite were utilized to identify the best-fit model with stringent adherence to the BMD modeling guidance (USEPA, 2000) regarding criteria for model fit (i.e., selection of the appropriate variance model, chi-square p-value criteria for model fits). The ED10 and ED10Low values were selected based upon the model with the "best fit" to the data according to the Akaike information criteria (AIC), p-value, and scaled residual of interest. The best fit values are shaded in the table. All modeling results are shown below:

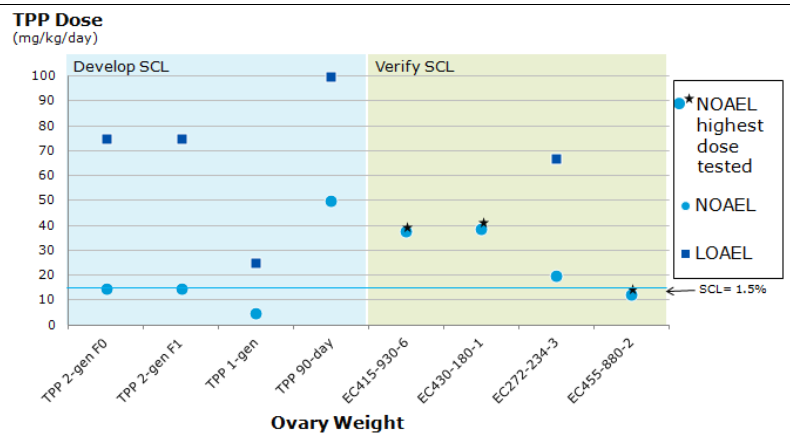
Continuous ED modeling of ovary weight in rats exposed to TPP

Study	Model Name	Goodness of Fit p-Value	AIC	Scaled Residual of Interest	ED_{101std} (mg/kg/day)	$ED_{10Lo1\ std}$ (mg/kg/day)
Knapp (2006) (1Gen) F_0 Females	**Exponential2**	0.2744	-763.89	-1.38	64.6	50.1
	Exponential3	0.2744	-763.89	-1.38	64.6	50.1
	Exponential4	0.6789	-764.30	-0.11	34.2	18.6
	Exponential5	N/A	-762.48	0.00	32.0	19.0
	Hill	N/A	-762.48	0.00	33.2	18.4

Ovary and Oviduct Weight	**Linear**	0.1894	-763.15	-1.56	71.8	58.0
	Polynomial	0.1894	-763.15	-1.56	71.8	58.0
	Power	0.1894	-763.15	-1.56	71.8	58.0
Edwards (2012) (2 Gen) F_0 Females Ovary Weight	**Exponential2**	0.7423	-814.69	0.34	33.0	25.3
	Exponential3	0.6013	-813.01	-0.12	38.1	25.7
	Exponential4	0.7423	-814.69	0.34	33.0	20.3
	Exponential5	N/A	-811.10	-0.05	18.1	15.3
	Hill	N/A	-811.10	-0.05	18.8	Failed
	Linear	0.8244	-814.90	0.12	36.3	28.9
	Polynomial	0.5723	-812.97	-0.10	39.8	29.0
	Power	0.5888	-813.00	-0.13	39.3	29.0
Edwards (2012) (2 Gen) F1 Females Ovary Weight	**Exponential2**	0.1477	-751.70	1.52	42.7	31.9
	Exponential3	0.3360	-752.60	0.00	71.0	39.6
	Exponential4	0.1477	-751.70	1.52	42.7	30.1
	Exponential5	N/A	-750.60	0.00	70.7	16.2
	Hill	N/A	-750.60	0.00	67.0	16.5
	Linear	0.2257	-752.55	-0.25	46.0	36.6
	Polynomial	0.6251	-754.58	0.00	64.3	40.8
	Power	0.3360	-752.60	0.00	72.1	40.9

As can be observed from the data within the table, the lower 95% confidence limits for ovary weight, for both the two-generation (Edwards, 2012) study (both generations) and the one-generation study (Knapp, 2006), result in ED10Low values greater than 1.5%. The value of 1.5% will enable appropriate classification for reproductive toxicity and avoid unwarranted classification of materials that contain insufficient TPP to pose a reproductive hazard. ED10Low values based upon a parameter of reproductive performance, litter size, resulted in higher values. These ED10Low values were 29 mg/kg/day (2.9%) for the Knapp 2006 study and 67.6 mg/kg/day (6.8%) for the Edwards 2012 study. For the Edwards 2012 study, only the final littering could be modeled because the earlier matings in the study did not result in litter sizes with significant differences from concurrent controls.

b. The SCL value of 1.5% is validated by existing test data for TPP-derived substances containing 2.5% to 26% TPP and tested at TPP-equivalent dosages of 0.125% to 6.7% (1.25 to 67 mg/kg/day). There were no effects upon TPP-responsive reproductive parameters in these studies at dosage levels below 1.5% (15 mg/kg/day). The figure below displays the NOAEL and LOAEL values for ovary weight determined from studies conducted with TPP (left side, blue), which were used to develop a NOAEL-based SCL value, and the analogous values determined for substances that contain TPP as an impurity (right side, green). For the substance identified as EC 455-880-2, the data point provided was the highest dose level tested.



It is unusual for verification data to be available for reproductive toxicity. However, the verification studies were conducted for substance registrations. No additional animal testing was performed.
i. The potency banding recommended in the ECHA guidance for SCL, based upon the ED10 methodology, is unwarranted for this substance in light of the following considerations:
a. The ED10Low values calculated from the two-generation (Edwards, 2012) reproduction study for TPP were compared to the ED10Low value for the supporting study, and the lowest value was utilized in setting the SCL. It is unusual to have two reproduction studies available, as provided herein, to provide reliable, consistent evidence of reproductive findings.

b. Data from reproduction studies conducted with substances that contain TPP provide adequate and conclusive confirmation that a reproductive hazard due to TPP does not exist at composition levels at or below 1.5%.

i. EC 415-930-6 was evaluated in a rat oral (gavage) two-generation reproduction study (Wood, 2002). At the test substance doses of 0, 50, 250 and 1000 mg/kg/day, the dose levels of TPP were 0, 1.9, 9.5, and 38 mg/kg/day. The parental NOAEL was 50 mg/kg/day (1.9 mg TPP/kg/day). The reproductive NOAEL was 250 mg/kg/day (9.5 mg/kg/day) based upon reductions to pregnancy index and litter size at 1000 mg/kg/day (38 mg TPP/kg/day). Ovary weight was not reduced in females of either generation, suggesting that the TPP-derived substance was of lesser potency for this effect.

ii. EC 430-180-1 was evaluated in a rat oral (gavage) two-generation reproduction study (Wood, 2003). At test substance doses of 0, 5, 30, or 150 mg/kg/day, the dose levels of TPP were 0, 1.3, 7.8, and 39 mg/kg/day. Ovary weight was not reduced in females of either generation. At 150 mg/kg/day (39 mg TPP/kg/day) female offspring achieved vaginal opening at a younger mean age (31.6 days versus 34.2 days) and lower average body weight in comparison to the concurrent control females. The NOAEL for vaginal patency was 30 mg/kg/day (7.8 mg TPP/kg/day).

iii. EC 272-234-3 was evaluated in a rat oral (gavage) two-generation reproduction study (Nemec, 1995). At test substance doses of 0, 50, 300, and 1000 mg/kg/day, the dose levels of TPP were 0, 3.4, 20.1, and 67 mg TPP/kg/day. Fertility and live litter size were reduced at 1000 mg/kg/day (67 mg TPP/kg/day); satellite groups that were cross-mated during the second generation (exposed males x unexposed females; unexposed males x exposed females) identified that these effects resulted from treatment of the female. Ovary weight was reduced at 1000 mg/kg/day (67 mg TPP/kg/day); the NOAEL for this parameter was 300 mg/kg/day (20.1 mg TPP/kg/day).

iv. EC 455-880-2 was evaluated in a rat oral (gavage) one-generation reproduction study (Knapp, 2008). At test substance doses of 0, 50, 170, and 500 mg/kg/day, the dose levels of TPP were 0, 1.25, 4.25, and 12.5 mg TPP/kd/day.

2. **Effects observed in organs of the male reproductive system may be secondary consequences of generalized systemic toxicity.** Additional support for the conclusion that effects observed to the rat male reproductive system are likely to be secondary to systemic health effects may be found by comparison of TPP results to results obtained from feed restriction studies in male Sprague-Dawley rats (Chapin et. al, 1993; Rehm et. al, 2008).

a. Feed restriction that produced similar magnitude changes to male body weights resulted in changes to male accessory reproductive organ weights (seminal vesicles, prostate) in a pattern similar to that observed in both of the TPP reproduction studies (Chapin et. al, 1993; Rehm et. al, 2008).

b. Testes weights are more resistant than accessory organs to reductions secondary to body weight decreases due to food restriction in adult male rats. This, too, was observed in the pattern of male reproductive effects due to TPP consumption.

c. Feed restriction did not adversely affect male reproductive performance (fertility) when body weight was reduced as low as 70% - 78% of concurrent control (Chapin, et. al 1993; Eng, et. al, 1987, respectively), although reduction of body weight to less than half of the concurrent control value resulted in significantly reduced fertility and litter size (Eng, et. al, 1987).

The data confirm similarities between the TPP and feed restriction studies regarding the magnitude of changes to body weight and accessory reproductive organ weights in male rats. This, we believe, provides sufficient reason to justifiably consider the most sensitive effects of TPP to the male reproduction system as secondary findings to systemic effects evidenced by reductions in body weight. We do not come to this conclusion regarding the most sensitive effects observed in female rats for this same study (see comment 4)

3. **The CLH report was submitted prior to issuance of the final report for the two-generation**
(Edwards, 2012) reproductive toxicity study, and does not include the interpretation of the independent laboratory. The CLH proposal by SI Group-UK, Ltd., references and is partially based upon, an earlier draft interpretation of the data from the study. The final, signed toxicology report cited as the two-generation (Edwards, 2012) study for classification is dated 11 May 2012 (author: Tammye L. Edwards). We request that ECHA utilize the interpretation of the toxicological findings as stated in the final report for your review of this CLH proposal. Toxicological clarifications stated in the final report include the following:

a. Page 132, Discussion: *"Tetrapropenyl phenol (TPP) administered at a dietary level of 75 mg/kg/day elicited a number of effects on body weights, food consumption, estrous cyclicity, reproductive performance, and developmental parameters. Adverse effects upon reproductive parameters were interpreted as direct effects upon the reproductive system and not a secondary consequence of generalized systemic toxicity."*

i. Regarding the reproductive performance of the first of the two-generations in this toxicology test (Section 6.2.5, page 77): *"No test substance-related effects on F0 reproductive indices were observed at any exposure level."* Slight alterations to reproductive

parameters, namely prolonged estrous cycles, reduced mean number of former implantation sites, and slightly smaller mean number of pups born, were observed at the highest dose level of 75 mg/kg/day.

ii. Regarding the reproductive performance of the second of the two-generations in this toxicology test (Section 6.3.5.2, page 109): *"The reduced fertility, copulation, and conception indices in the 75 mg/kg/day group corresponded to the decreased number of corpora lutea in this group that was observed microscopically (see Section 6.3.8.3). Therefore, the lower fertility, copulation, and conception indices in the 75 mg/kg/day group during the second mating cycle were considered the adverse result of test substance exposure."* During the second generation phase of the study, an unexplained non-dose-responsive decline in fertility occurred in all groups including the control animals; the ability of the data to detect intergroup differences was questioned. For this reason, the adult rats (F1) were mated a second time after the initial litters were weaned. The above quotation from the study refers to the second mating of the F1 test subjects. The difference in the mean value for the number of pups born, 13.4 pups born per litter in the control group versus 10.1 pups born per litter in highest exposure group (75 mg/kg/day) was statistically significant.

b. It is suggested that the data accessible through the ECHA Chemical Substance Search portal be updated with the interpretation of the study director and testing laboratory.

4. Effects upon reproductive parameters in females do not appear to be secondary to generalized systemic toxicity.

a. A comparison of TPP results to results obtained from feed restriction studies in female Sprague-Dawley rats (Chapin et. al, 1993; Seki et. al, 1997) suggests that female body weight effects which occurred in the TPP reproduction studies were insufficient to account for the effects upon estrous cyclicity or reductions in corpora lutea, implantation sites, or pup litter size.

i. In both the two-generation and one-generation TPP rat reproduction studies, doses of 75 mg/kg/day and 125 mg/kg/day, respectively, lengthened estrous cycles in association with a prolonged diestrus phase. In the two-generation study, this occurred concurrently with reduced body weight compared to the concurrent control groups. However, the degree of difference from control body weight at the end of the premating phases, 12.6% to 12.5% for each parental generation, is insufficient to account for the alterations to female cyclicity. Feed restriction studies by both Chapin et. al (1993) and Seki et. al (1987) demonstrated that the female Sprague-Dawley rat will display such effects when body weight is attenuated by 30% below the control group weight, but these effects did not occur when the severity was no more than 20% reduced from control body weight.
The supporting reproduction study (Knapp, 2006) also identified changes to these reproductive endpoints in the presence of reduced female body weight that was 90% of concurrent control at the time of mating. This study, in combination with the findings of the two-generation study, suggest both a threshold for adverse effects upon reproductive performance in females and a steep dose-response curve, as effects upon reproductive performance occurred minimally at 75 mg/kg/day and became severe at 125 mg/kg/day. The most sensitive, reproducible, statistically significant measurement among female reproductive parameters was reduced ovary weight at 25 mg/kg/day. This occurred in the absence of additional statistically significant effects upon fertility.
ii. Mechanistic studies performed with female pubertal or ovariectomized female rats indicate that TPP exposure can affect the female reproductive organs.
1. Oral administration of TPP to pubertal female rats (PND 22-41) accelerated the onset of vaginal opening and lowered ovary weight (NOAEL 10 mg/kg/day; LOAEL 50 mg/kg/day),

with changes to ovarian morphology and the onset of first estrus at 200 mg/kg/day (Knapp, 2009a). Eight of fifteen animals died at 800 mg/kg/day.
2. Oral administration of TPP (75 – 500 mg/kg/day) to ovariectomized female rats (ca. 60 days of age at initiation) for three days resulted in increased uterine wet and blotted weights in a dose-responsive manner (Edwards, 2010a).
3. Steroid receptor binding assays indicated that TPP is a weak competitive ligand for the estrogen receptor (IC50 = 1100 nM relative to estradiol IC50 = 1.2 nM; receptor binding affinity of 0.1) and androgen receptor (IC50 = 9200 nM relative to R1881 IC50 = 1.44 nM; receptor binding affinity of 1.57 x 10-7; Thomas 2012a, b)

References

Chapin, RE, Gulati, DK, Barnes, LH, Teague, JL (1993) The effects of feed restriction on reproductive function in Sprague-Dawley rats. Fundamental and Applied Toxicology 20: 23-29.
Crump, KS. (1995). Calculation of benchmark doses from continuous data. Risk Analysis 15:79-89.
Edwards, TL (2012) A dietary two-generation reproductive toxicity study of tetrapropenyl phenol in rats. WIL Research Laboratories, LLC, Study No. WIL-186053; conducted for the American Chemistry Council.
Edwards, TL (2010a) A uterotrophic assay of tetrapropenyl phenol administered orally in ovariectomized rats. WIL Research Laboratories, LLC, Study No. WIL-187092; conducted for Chevron Energy Technology Company.
Eng, LA, Lee, JH, Sponenberg, DP (1987) Food restriction in adult male rats: effects on mating, pregnancy, litter size and offspring growth rate. Nutrition Research 7: 957-963.
Knapp, JF (2006) An oral (gavage) one-generation reproductive toxicity study of tetrapropenyl phenol in rats. WIL Research Laboratories, LLC, Study No. WIL-186033; conducted for the American Chemistry Council.
Knapp, JF (2008) An oral (gavage) one-generation reproductive toxicity study of EC 455-880-2 in rats. WIL Research Laboratories, LLC, Study No. WIL-187045; conducted for Chevron Energy Technology Company.
Knapp, JF (2009) A female pubertal assay of CAS RN 74499-35-7 administered orally in juvenile female rats. WIL Research Laboratories, LLC, Study No. WIL-187060; conducted for Chevron Energy Technology Company.
Nemec, MD (1995) A two-generation reproductive toxicity study of EC 272-234-3 in rats. WIL Research Laboratories, LLC, Study No. WIL-187006; conducted for Chevron Energy Technology Company.
Rehm, S, White, TE, Zahalka, EA, Stanislaus, DJ, Boyce, RW, Wier, PJ (2008) Effects of food restriction on testis and accessory sex glands in maturing rats. Toxicological Pathology 36: 687-694.
Seki, M, Yamaguchi, K, Marumo, H, Imai, K. (1997). Effects of Food Restriction on Reproductive and Toxicological Parameters in Rats: In Search of Suitable Feeding Regimen in Long-Term Tests, Toxicological Sciences, 22, pp 427 - 437.
Thomas, J (2012a) In vitro rat prostate androgen receptor competitive binding assay with tetrapropenyl phenol. WIL Research Laboratories, LLC, Study No. WIL-187133; conducted for Chevron Energy Technology Company.
Thomas, J (2012b) In vitro rat uterine estrogen receptor competitive binding assay with tetrapropenyl phenol. WIL Research Laboratories, LLC, Study No. WIL-187137; conducted for Chevron Energy Technology Company.
U.S. Environmental Protection Agency (USEPA). (2000). Benchmark dose technical guidance document. EPA/630/R-00/001.
Wood, E (2002) EC 415-930-6: Oral gavage two generation reproduction study in the rat. SafePharm Laboratories, Study No. 703/224R; conducted for Chevron Energy Technology Company.

Wood, E (2003) EC 430-180-1: Oral gavage two generation reproduction study in the rat. SafePharm Laboratories, Study No. 703/256; conducted for Chevron Energy Technology Company.

Appendix. Study Summaries

I. Assays Conducted with TPP

a. Female Pubertal Assay

b. Uterotrophic Assay

c. Receptor Binding Assay (*In Vitro Rat Prostate Androgen Receptor)*

d. Receptor Binding Assay (*In Vitro Rat Uterine Estrogen Receptor)*

II. Reproduction Studies Conducted with TPP-Derived Substances

a. EC 415-930-6

b. EC 430-180-1

c. EC 272-234-3

d. EC 455-880-2

I. Assays Conducted with TPP

(a) *Knapp, JF. 2009. A female pubertal assay of CAS RN 74499-35-7 Administered Orally in Juvenile Female Rats. WIL Research Laboratories, LLC, Study No. WIL-187060.Conducted for Chevron Energy Technology Company.*

Study Design

TPP, in the vehicle, corn oil, was administered orally by gavage once daily for 20 consecutive days to four groups each of 15 Crl:CD(SD) immature female rats. Dosage levels were 10, 50, 200 and 800 mg/kg/day, and the dosage volume was 5 mL/kg. A concurrent control group received the vehicle on a comparable regimen.

Summary of No Observed Adverse Effect Levels End Point	**NOAEL (mg/kg bw/day)**
Vaginal Patency (Female)	10
Ovary Weight (Female)	10

Results

Mortality

Eight of fifteen (8/15, 53%) females in the 800 mg/kg/day group died following one to four days of exposure. Because this significantly exceeded the maximum tolerated dose, data from this group are not reported.

Body Weight Changes

Mean body weight gain in the 200 mg/kg/day group was lower (not statistically significant) than the control group value (PND 22-42).

Mean body weights and body weight gains in the 10 and 50 mg/kg/day groups were generally similar to those in the control group throughout the study (no statistically significant differences).

Vaginal Opening

Accelerated (early) vaginal opening (VO) observed at 50 and 200 mg/kg/day ($p < 0.01$)

o Mean day of attainment of vaginal opening was 32.5, 33.3, 28.3, and 28.2, days in the control, 10, 50, and 200 mg/kg/day groups, respectively (historical control range of 31.8 – 36.5 days).

o *Note: acceleration was not observed in* F1 *offspring in the one-generation reproduction gavage study at 25 mg/kg/day (Knapp, 2006); exposure post-weaning (intentionally not*

conducted in the 1-generation study) may be necessary to alter puberty timing.
Decreased body weight at vaginal opening at 50 and 200 mg/kg/day ($p < 0.01$)
o Mean body weights (g) were 85.4 and 83.4g at attainment across these dose groups versus 111.9 g in the control group.

Estrous Cycle
Mean first occurrences of estrus were earlier at 50 and 200 mg/kg/day (32.1 and 31.2 days, respectively) than in the control group (34.4 days). The difference was statistically significant ($p<0.05$) in the 200 mg/kg/day. At 200 mg/kg/day, 12/15 females exhibited extended estrus (> 3 consecutive days).

Note: Estrous cycle lengths could only be determined for 5/15, 8/15, 13/15, and 7/15 females in the control, 10, 50, and 200 mg/kg/day groups, respectively, due to a high number of females with incomplete cycles. No differences in estrous cycle lengths were noted in the 10, 50 and 200 mg/kg/day groups with number of cycling animals available for evaluation. Estrous cycle lengths in females of this age are highly variable. Abnormal estrous cycles (≥3 consecutive days estrous [E]) were noted in 12/15 females in the 200 mg/kg/day group.

Hormone Effects
No changes to TSH or T4 in this study at any dosage level.
No changes to E2 and LH in this study at any dosage level.

Histological Effects
Decreased corpora lutea in the ovaries of the 200 mg/kg/day group animals (incidence of 0/15 in the control group vs. 9/15 in the 200 mg/kg/day group)
o Granulosa cell necrosis in the ovaries of the 200 mg/kg/day group animals (incidence of 0/15 in the control group vs. 15/15 in the 200 mg/kg/day group)
o Oocyte degeneration in the ovaries of the 200 mg/kg/day group animals (incidence of 0/15 in the control group vs. 15/15 in the 200 mg/kg/day group)

Organ Weights
Statistically significant reduction ($p<0.05$ and $p<0.01$) in mean absolute ovary weights in the 50 and 200 mg/kg/day groups.
Ovary weight (g) with oviducts were 0.0936, 0.0855, 0.0787, 0.0548, g across the dose groups (0, 10, 50, 200 mg/kg/day, respectively)
Statistically significant reduction ($p<0.01$) in mean absolute and relative thymus weights in the 200 mg/kg/day group
Statistically significant reduction ($p<0.01$) in mean absolute and relative uterus (wet and blotted) weights in the 200 mg/kg/day group
Uterus – wet (g) was 0.4201, 0.3760, 0.4013, 0.2177 g across the dose groups.
Uterus – blotted (g) was 0.3371, 0.3277, 0.3083, 0.2022g across the dose groups

Other Findings
Eight females in the 800 mg/kg/day were found dead at 23, 25 or 26 days of age as a result of test article administration. Three females in this group were found dead at approximately 1-2 hours following dose administration; the remaining 5 females were found dead on the morning following their last dose.

Discussion and Conclusions

Female Reproductive Toxicity
From the results, it can be concluded that TPP administered orally to juvenile female rats

resulted in estrogenic effects for females at 50 and 200 mg/kg/day as evidenced by earlier attainment of vaginal patency (with corresponding lower mean body weight on the day of attainment) and at 200 mg/kg/day by earlier age at the first occurrence of estrus. Animal deaths attributed to the 800 mg/kg/day dose level in this study were a result of elevated test article administration dose levels.

Estrous cycle disturbances were noted in the 200 mg/kg/day group with 12/15 females exhibiting persistent estrus (≥3 consecutive days of estrus). No test article-related effects on mean serum E2, LH, T4 or TSH levels were observed at any dosage level. Mean absolute and relative wet and blotted uterusweights (and thus, luminal fluid weight) and thymus gland weights in the 200 mg/kg/day groups were lower than the control group values. Lower mean absolute ovary/oviduct weights were observed in the 50 and 200 mg/kg/day groups. In the 200 mg/kg/day group, morphologic changes (absent corpora lutea, oocyte degeneration, granulose cell necrosis) in ovaries were present.

(b) Edwards, T.L. et al., 2010a. An uterotrophic assay of tetrapropenylphenol administered orally in ovariectomized rats. Report for WIL Research Laboratories, LLC, Study No. WIL-187092, Conducted for Chevron Energy Technology Company.

Study Design
The test substance, tetrapropenyl phenol, in the vehicle, corn oil, was administered orally by gavage to four groups of six ovariectomized female Crl:CD(SD) rats once daily for three consecutive days in accordance with OECD 440 test guidelines (2007). Dosage levels were 75, 125, 250, and 500 mg/kg/day (dose volume of 5ml/kg). A positive control group (Group 2) composed of six ovariectomized females received the estrogenic positive control agent (17α-ethynylestradiol) orally by gavage in corn oil at a dosage level of 0.2 mg/kg/day. A concurrent vehicle control group (Group 1) composed of six ovariectomized females received the vehicle (corn oil) on a comparable regimen. The dosage volume was 5 mL/kg for all groups. The females were approximately 42 days of age at the time of ovariectomy (performed by the the supplier) and approximately 60 days of age at the beginning of test substance administration.

Summary of No Observed (Adverse) Effect Levels

End Point	**1 NO(A)EL (mg/kg bw/day)**
Weight Changes (Female)	< 75 - NOEL
Uterine Weights (Female)	< 75 - NOAEL

Results
Body Weight Changes
Decreased body weight gain observed at all dose levels, including the positive control. Mean body weights were 5.5%, 6.6%, 7.0%, and 11.1% lower in the 75, 125, 250, and 500 mg/kg/day groups, respectively, compared to the vehicle control group on study day 3. In the positive control group, mean body weight losses were noted throughout the treatment period, resulting in a 10.7% lower mean body weight compared to the vehicle control group on study day 3.

Macroscopic Examinations
At the scheduled euthanasia, no internal findings were observed in the uterus at any dosage level.

Uterine Weights
Dose-related increase in absolute and relative uterine wet and blotted at all doses.

o Blotted: 183% to 275% of control value
o Wet: 181% to 739% of control value

Discussion and Conclusions

Female Reproductive Toxicity
Oral gavage administration of TPP to ovariectomized rats at dosages of 75, 125, 250, and 500 mg/kg/day for three days resulted in dose-dependent increases in wet and blotted mean uterine weights at all exposure levels compared to the vehicle control group. The positive control substance (17α-ethynylestradiol) also elicited the expected increase to uterine weights (wet and blotted). The interpretation of the laboratory was that TPP "demonstrated or mimicked biological activities consistent with agonism of natural estrogens".

(c) Thomas, J., et. al. 2012a. In Vitro Rat Prostate Androgen Receptor Competitive Binding Assay with Tetrapropenyl Phenol. WIL Research Laboratories, Study number WIL-187133. Conducted for Chevron Energy Technology Company.

Experimental Procedures
The objective of this study was to evaluate the ability of the test substance, TPP, to inhibit the binding of a radiolabeled ligand, 3H-R1881, to the androgen receptor (AR). The androgen receptor is responsible for key steps in the development of male sexual characteristics. The study was designed and executed in compliance with Endocrine Disruptor Screening Program Test Guidelines OPPTS 890:1150: Androgen Receptor Binding (Rat Prostate Cytosol).
Thirty male Sprague-Dawley Crl:CD(SD) rats were obtained for use in this study from Charles River Laboratories (Raleigh, NC). Each animal was castrated approximately 24 hours before euthanasia to allow the endogenous concentrations of DHT and testosterone (a 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.
Following a 24-hour incubation of the ligand with the receptor, the specific binding of the radiolabeled ligand to the AR at equilibrium was determined. Bound ligand was separated from unbound ligand with hydroxyapatite. A saturation binding assay was conducted to determine the density of functional ARs in the cytosol preparation (Bmax) as well as the dissociation constant (Kd) for binding of the 3H-R1881 ligand to the AR.
Prostate cytosol was used to demonstrate the response of the test system to a known weak positive control compound (dexamethasone) and to evaluate the test substance for the ability to inhibit R1881 binding. Test substance and positive control compound concentrations spanned a range of 0.1 nM to 1 mM. The dose-response curves were evaluated to determine if competitive binding was present. IC50 values were determined as the inflection point for the sigmoidal dose-response curve.

Summary
The AR assay combines rat prostate cytosol containing the AR, radiolabeled R1881 (ligand), and the test substance. The effect of the varying test substance concentrations on R1881 binding is evaluated by measuring the amount of ligand displaced by increasing concentrations of the test substance. The AR binding assay is thus conducted over a range of test substance concentrations such that a dose responsive curve can be developed if R1881 binding is affected by the presence of the test substance.
Preliminary assays were performed to ensure androgen receptor (AR) concentration and

specificity in the prepared rat prostate cytosol. Rat prostate homogenate showed sufficient activity and acceptable affinity for the non-labeled ligand during saturation binding assays. Once the rat prostate cytosol met the acceptance criteria in the protocol, the assay was used to evaluate TPP for the ability to bind to the AR.
Concurrent analysis of a positive control chemical (non-labeled R1881) and a weak positive control chemical (dexamethasone) confirmed acceptable assay performance. The mean TPP response curve indicated that TPP had the ability to disrupt ligand binding starting at an approximate concentration of 10 µM and resulting in complete inhibition by 1 mM TPP (mean daily % 3H-R1881 bound <15%). The response curve demonstrated the expected shape of a normal inhibition curve over 2 log units of concentration and fit the 4-parameter nonlinear regression model. A mean IC50 of approximately 92 µM was determined for TPP inhibition of R1881 binding. The binding affinity of TPP was similar to the weak positive control, dexamethasone.
TPP was considered an androgen receptor binder according to the data interpretation criteria in the protocol and the EPA guidance document.

Relative Binding Affinity (RBA)
As suggested by NIH Publication No. 03-4506, the potential for variation in IC50 values among AR binding assays warrants comparison by an additional metric, namely the %RBA (Competitive Binding Assay Data Analysis). %RBA values were generated by comparing the arithmetic mean inter-assay IC50 values for test and reference compounds.
The %RBA observed for all test chemicals exhibited the following order:
[R1881 > Dexamethasone > Test Material]
The order of AR binding for the first 2 chemicals shown above was in agreement with the test guideline. The % RBA for TPP was 1.57 × 10-7%.

Conclusion
Rat prostate cytosol was successfully prepared and characterized, then employed in competition assays with the goal of investigating the potential of TPP to act as an endocrine disruptor. Results from these experiments indicate that TPP binds to the active site in a competitive manner with R1881 and is considered an androgen receptor binder according to the data interpretation criteria in the protocol and the EPA guidance document.

(d) Thomas, J., et. al. 2012b. In Vitro Rat Uterine Estrogen Receptor Competitive Binding Assay with Tetrapropenyl Phenol. WIL Research Laboratories, Study number WIL-187137. Conducted for Chevron Energy Technology Company.

Study Design
The objective of this study was to evaluate the ability of the test substance TPP to inhibit the binding of a radio-labeled ligand, hexatritiated 17β-estradiol, to the estrogen receptor (ER). The estrogen receptor is responsible for controlling the production of mRNA and is critical in regulating key steps in the development of female sexual characteristics. The study was designed and executed in compliance with Endocrine Disruptor Screening Program Test Guidelines OPPTS 890:1250: Estrogen Receptor Binding using Rat Uterine Cytosol (ER-RUC).

Thirty female Sprague-Dawley Crl:CD(SD) rats were obtained for use in this study from Charles River Laboratories (Portage, MI). Each animal was ovariectomized approximately 9 days before euthanasia. 18

Immediately following euthanasia, the uterine tissue was pooled and homogenized, followed by centrifugation to collect the cytosolic fraction containing the ER. 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 ER concentration. Following a 16-20 hour incubation of the ligand with the receptor, the specific binding of the radio-labeled ligand to the ER at equilibrium was determined. Bound ligand was separated from unbound ligand with hydroxyapatite. A saturation binding assay was conducted to determine the density of functional ERs in the cytosol preparation (Bmax), as well as the dissociation constant (Kd) for binding of the 3H-E2 ligand to the ER.

Uterine cytosol was used to demonstrate the response of the test system to a known weak positive control compound (non-labeled estradiol), a weak positive control chemical (19-norethindrone), and a negative control chemical (octyltriethoxysilane) and to evaluate the test substance ability to inhibit 3H-E2 binding. Test substance and positive control compound concentrations spanned a range of 0.1 nM to 0.1 mM. The dose-response curves were evaluated to determine if competitive binding was present. IC50 values were determined as the inflection point for the sigmoidal dose-response curve.

Summary
The ER assay combines rat uterine cytosol containing the ER, radio-labeled estradiol (ligand), and the test substance TPP. The effect of the varying test substance concentrations on estradiol binding was evaluated by measuring the amount of radio-ligand displaced by increasing concentrations of the test substance. The ER binding assay was thus conducted over a range of test substance concentrations such that a dose responsive curve can be developed if estradiol binding was affected by the presence of the test substance. The inhibitory concentration at which 50% of the radio-ligand is displaced (IC50) was determined from the dose-response curve.
Preliminary assays were performed to ensure estrogen receptor (ER) concentration and specificity in the prepared rat uterine cytosol. Rat uterine homogenate met the acceptance criteria for sufficient affinity for the non-labeled ligand during saturation binding assays. Once the rat uterine cytosol met the acceptance criteria in the protocol, the assay was used to evaluate the ability of TPP to bind to the ER.
Concurrent analysis of a positive control chemical (non-labeled estradiol) and a weak positive control chemical (19-norethindrone), and a negative control chemical (octyltriethoxysilane) confirmed acceptable assay performance. TPP had the ability to disrupt ligand binding starting at an approximate concentration of 10-7 M and resulting in complete inhibition at a concentration of 10-5 M. A mean inter-day IC50 for TPP was approximately 1100 nM.

Relative Binding Affinity
As suggested by NIH Publication No. 03-4506, the potential for variation in IC50 values among AR binding assays warrants comparison by an additional metric, namely the %RBA (Competitive Binding Assay Data Analysis). %RBA values were generated by comparing the arithmetic mean inter-assay IC50 values for test and reference compounds:

$$\%\,\mathrm{RBA} = \frac{IC_{50}\ (\text{un-labeled estradiol})}{IC_{50}\ (\text{Competitor})} \times 100$$

The %RBA observed for all test chemicals exhibited the following order:
[un-labeled Estradiol > 19-Norethindrone > Test Substance TPP]

The arithmetic mean IC50 for the reference was found to be 1.2 nM. This is close to the expected IC50 of 1.4 nM, as calculated by summing the concentration of radio-ligand (1 nM) and its affinity for the ER (Kd, 0.41 nM). The arithmetic calculation of the mean IC50 for 19-norethindrone (weak positive control) resulted in a value of 3.46 µM and a %RBA of

0.034%. Octyltriethoxysilane (0.1 nM to mM) was used as the negative control compound in this study. As such, no interaction with the ER was expected. The calculated IC50 for TPP was 1100 nM and the % RBA for test substance TPP was 0.11%.

Conclusion
Rat uterine cytosol was successfully prepared and characterized, then employed in competition assays with the goal of investigating the potential of TPP to act as an endocrine disruptor. Results from these experiments indicate that TPP is a possible ligand for the rat ER, and the mean response curve indicated that TPP was able to disrupt ligand binding. Therefore, TPP is considered interactive with the estrogen receptor. The mean inter-day IC50 was approximately 1100 nM, and the %RBA relative to the reference estradiol ligand was 0.1.

II. Reproduction Studies Conducted with TPP-Derived Substances
(a) Wood, E. (2002) [EC415-930-6]: Oral gavage two generation reproduction study in the rat. SafePharm Laboratories, Ltd., SPL Project No. 703/224R. Conducted for Chevron Energy Technology Company.

Study Design
The study was originally intended as a modified OECD 415 (the test design of the first generation of the OECD 416), but was extended to a second generation and met the OECD 416 requirements except that anogenital distance was not measured in the second generation offspring. Parental generations were identified as F0 and F1. The progeny of the F0 animals were identified as the F0a (pups) until selected and identified as F1 for the second generation and F0b (fetuses). The progeny of the F1 animals were identified as the F2 (pups).
Test Substance: EC415-930-6 (3.8wt% TPP)
Test Animals: Sprague-Dawley Crl:DC IGS BR Rats; N = 28/sex/group (F0)
Dose levels: 0 (corn oil), 50, 250, 1000 mg/kg/day (equivalent to 0, 1.9, 9.5, and 38 mg TPP/kg/day)
Dosing period: Daily, at least ten weeks prior to mating for adults (both sexes, both generations), throughout mating, gestation and lactation; F1 offspring selected to form the second parental generation or retained for determination of sexual maturation (vaginal opening, preputial separation) began direct dosing after weaning.

Summary of No Observed Adverse Effect Levels

End Point/Generation	NOAEL (mg/kg/day)
Parental (F_0, F_1)	50 (1.9 mg TPP/kg/day)
Reproduction (F_0, F_1)	250 (9.5 mg TPP/kg/day)
Offspring (F_{0a}, F_2)	50 (1.9 mg TPP/kg/day)

Summary of Results Consistent with TPP Reproductive Toxicity
(Note: all findings consistent with reproductive effects of TPP, whether attributed to test substance exposure or not attributed, are shown below; study director justification included as appropriate. There were no effects to ovary weight, estrous cyclicity, implantation numbers, mean value for corpora lutea, or age/weight at vaginal opening)

1000 mg/kg/day (38 mg TPP/kg/day)
- low incidence of females with absent corpora lutea (F1 only)
- decreased pregnancy rate (F0, F1)
o F0a phase: 77.8% vs. 100% control
o F0b phase: 80.8% vs. 96.4% control
o F1 phase: 84.0% vs. 96.4% control

- decreased mean liver litter size
o F0a: 12.0 vs. 13.5 (control); not statistically significant
o F2: 12.3 vs. 14.4 (control); statistically significant
- decreased epididymal/body weight ratio at body weight of ca. 90% control value at termination
o F0: 0.1060 vs. 0.1159 control (left); 0.1107 vs. 0.1257 control (right)
o F1: no effect

250 mg/kg/day (9.5 mg TPP/kg/day) – not attributed by the study director to test substance administration due to the absence of replication in the F1 mating phase. Three out of the four males that failed to produce a litter were found to have testicular epithelial degeneration or an absence of germinal epithelium. This was not observed in any of the F1 exposure groups.
- decreased pregnancy rate
o F0a phase: 71.4% vs. 100% control
o F0b phase: 75.0% vs. 96.4% control
o F1 phase: no effect

50 mg/kg/day (1.9 mg TPP/kg/day) – not attributed by the study director to test substance administration due to the absence of observation in the F0a phase or replication in the F1 mating phase.
- decreased pregnancy rate
o F0a phase: 92.8%% vs. 100% control – within normal pregnancy rate range
o F0b phase: 82.1% vs. 96.4% control
o F1 phase: no effect

Additional Reproductive Findings 1000 mg/kg/day (38 mg TPP/kg/day)
- dystocia
o F0a phase: 1 female/20 (littered)
o F0b phase: not applicable (terminated gestation day 20)
o F1 phase: 4 females/ 15 (littered)
- delayed balano preputial separation in males
o F0a phase: 44 days vs. 43 days control; not statistically significant
body weight at attainment: 220 g vs. 201 g; statistically significant

250 mg/kg/day (9.5 mg TPP/kg/day)
- delayed balano preputial separation in males
o F0a phase: 45 days vs. 43 days control; statistically significant
body weight at attainment: 216 g vs. 201 g; statistically significant

50 mg/kg/day (1,9 mg TPP/kg/day)
- delayed balano preputial separation in males
o F0a phase: 44 days vs. 43 days control; not statistically significant
body weight at attainment: 215 g vs. 201 g; not statistically significant

Summary of Parental Toxicity Findings

Males
1000 mg/kg/day (38 mg TPP/kg/day): decreased body weight (90% of control in F0; 93% of control in F1); sporadic increase in food consumption (F0, F1) altered hematology (increased activated partial thromboplastin time, and reduced red cell count); altered serum chemistry (increased alkyline phosphatase), increased weights of kidney, spleen, liver, and/or adrenal; no microscopic changes attributed to treatment

250 mg/kg/day (9.5 mg TPP/kg/day): altered hematology (increased activated partial thromboplastin time, and reduced red cell count); altered serum chemistry (decreased creatinine); increased weights of kidney, liver (F0 only); no microscopic changes attributed to treatment
50 mg/kg/day (1.9 mg TPP/kg/day): no effects attributed to treatment

Females
1000 mg/kg/day (38 mg TPP/kg/day): altered hematology (increased clotting time); altered serum chemistry (increased alanine aminotransferase, blood urea, phosphorus, and calcium); increased weights of liver and adrenals; no microscopic changes attributed to treatment
250 mg/kg/day (9.5 mg TPP/kg/day): no effects attributed to treatment
50 mg/kg/day (1.9 mg TPP/kg/day): no effects attributed to treatment

Conclusions
Adverse effects consistent with TPP reproductive toxicity included decreased pregnancy rates and litter sizes in both generations of the study and reduced relative epididymal weight at 1000 mg/kg/day (38 mg TPP/kg/day). The reduction in pregnancy rate that was observed at lower doses during the first or second mating of the F0 generation was not replicated in the second generation and was not attributed to exposure. There were no reproductive effects consistent with the profile of TPP toxicity at 250 or 50 mg/kg/day (9.5 and 1.9 mg TPP/kg/day, respectively). There were no effects to other parameters of TPP toxicity at any dose level.

In this study, other reproductive effects were observed that were attributed to treatment but are inconsistent with the profile of TPP reproductive toxicity. These effects included dystocia and delayed sexual maturation in males (not dose-responsive). For the latter finding, a subsequent study conducted at the same laboratory with the same strain of test animals obtained a similar finding but had a higher control value, and the study director commented upon the variability of body weight and age observed for balano preputial separation.

(b) Wood, E. (2003) [EC430-180-1]: Oral gavage two generation reproduction study in the rat. SafePharm Laboratories, Ltd., SPL Project No. 703/256. Conducted for Chevron Energy Technology Company.

Study Design
The study was originally intended as a modified OECD 415 (the test design of the first generation of the OECD 416), but was extended to a second generation and met the OECD 416 requirements except that anogenital distance was not measured in the second generation offspring. Parental generations were identified as F0 and F1. The progeny of the F0 animals were identified as the F0- F1 (pups) until selected and identified as F1 for the second generation. The progeny of the F1 animals were identified as the F1-F2 (pups).
Test Substance: EC430-180-1 (26wt% TPP)
Test Animals: Sprague-Dawley Crl:DC IGS BR Rats; N = 28/sex/group (F0)
Dose levels: 0 (corn oil), 5, 30, 150 mg/kg/day (equivalent to 0, 1.3, 7.8, 39 mg TPP/kg/day)

Dosing period: Daily, at least ten weeks prior to mating for adults (both sexes, both generations), throughout mating, gestation and lactation; F1 offspring selected to form the second parental generation or retained for determination of sexual maturation (vaginal opening, preputial separation) began direct dosing after weaning.

Summary of No Observed Adverse Effect Levels

End Point/Generation	NOAEL (mg/kg/day)
Parental (F_0, F_1)	< 5 (<1.3 mg TPP/kg/day)
Reproduction (F_0, F_1)	150 (39 mg TPP/kg/day)
Offspring (F_0-F_1, F_1-F_2)	30 (NOEL) (1.9 mg TPP/kg/day)

Summary of Results Consistent with TPP Reproductive Toxicity

(note: all findings consistent with reproductive effects of TPP, whether attributed to test substance exposure or not attributed, are shown below; study director justification included as appropriate. There were no effects upon ovary weight, estrous cyclicity, or pregnancy weight)

150 mg/kg/day (39 mg TPP/kg/day)

- decreased mean liver litter size

o F0-F1: 13.0 vs. 14.4 (control); not statistically significant

o F1-F2: no effect

- decreased mean uterine implantation sites

o F0: 13.7 vs. 15.2 (control); not statistically significant

o F1: no effect

- earlier onset of sexual maturation in females (vaginal opening)

o F0-F1: 31.6 days vs. 34.2 days (control)

☐ body weight at attainment: 92 g vs. 107 g (control

o F1-F2: not evaluated

- decreased epididymal weight ratio at body weight of ca. 95% control value at termination

o F0: 0.683 g vs. 0.738 g control (left)

o F1: no effect

- decreased seminal vesicle/coagulating gland weight (absolute and relative to body weight)

o F0: 2.335 g vs. 2.726 g (control); 0.384 vs. 0.435 (control)

o F1: 2.261 g vs. 2.761 g (control); 0.392 vs. 0.447 (control)

Additional Reproductive Findings

(note: With TPP, reductions to accessory organs of the male reproductive system occurred concurrently with reduced body weight. In this study conducted with EC 430-180-1, accessory male organ weights were reduced in the absence of concurrent body weight reductions. For this reason, these effects, when not observed with body weight changes, are listed here under Additional Reproductive Findings. It seems logical that the reduction in accessory male reproductive organ weight at all dosage levels is likely by a similar/same mechanism of action, but we did not want to exclude any findings that have occurred with TPP. EC 430-180-1 contained many other constituents. It is no longer in commercial usage.)

150 mg/kg/day (39 mg TPP/kg/day)

- increased mean pituitary/body weight ratio

o F0: no effect

o F1: 0.003 vs. 0.002 (control)

- slight changes to the proportion of growing and antral follicles (deemed by the study director as within biological variability, but included for completeness)

o F0: no effect

o F1: medium follicles, 0.31 vs. 1.0 (control); large follicles, 0.5 vs. 1.0 (control)

- increased homogenization resistant spermatid count in testis

o F1: 77.5 vs. 71.2 (control) (in millions)

- delayed balano preputial separation in males

o F0-F1: 44.7 days vs. 43.8 days (control)

☐ body weight at attainment: 214 g vs. 200 g (control)

o F1-F2: not evaluated

30 mg/kg/day (7.8 mg TPP/kg/day)

- decreased seminal vesicle/coagulating gland weight (absolute and/or relative to body weight)
o F0: 2.480 g vs. 2.726 g (control); 0.402 vs. 0.435 (control)
o F1: 2.371 g vs. 2.761 g (control); 0.4991 vs. 0.447 (control)
- decreased epididymal weight
o F0: 0.687 g vs. 0.738 g (control); (left0
o F1: no effect

5 mg/kg/day (1.3 mg TPP/kg/day)
- decreased seminal vesicle/coagulating gland weight (absolute or relative to body weight)
o F0: 0.394 vs. 0.435 (relative)
o F1: 2.434 g vs. 2.761 g (control) (absolute)

Summary of Parental Toxicity Findings

Males
150 mg/kg/day (39 mg TPP/kg/day): decreased body weight (ca. 95% of control); increased organ weights for liver, kidneys; no microscopic changes attributed to treatment
30 mg/kg/day (7.8 mg TPP/kg/day): increased thyroid weight (F1); no microscopic changes attributed to treatment
5 mg/kg/day (1.3 mg TPP/kg/day): increased thyroid weight (F1); no microscopic changes attributed to treatment

Females
150 mg/kg/day (39 mg TPP/kg/day): no effects attributed to treatment
30 mg/kg/day (7.8 mg TPP/kg/day): increased thyroid/body weight ratio (F0); decreased liver weight (absolute and relative to body weight)(F1); no microscopic changes attributed to treatment
5 mg/kg/day (1.3 mg TPP/kg/day): increased thyroid/body weight ratio (F0); no microscropic changes attributed to treatment

Conclusions
Adverse effects consistent with TPP reproductive toxicity included slight, not statistically significant reductions to litter size and implantation number and accelerated female sexual maturation at 150 mg/kg/day (39 mg TPP/kg/day) and reductions in epididymal and seminal vesicle/coagulating gland weights in the presence of a reduction in male body weight gain.

In this study, other reproductive effects observed but inconsistent with the profile of TPP reproductive toxicity included decreases in male accessory reproductive organ weights in the absence of body weight changes, delayed sexual maturation in male offspring, increased homogenization-resistant spermatid count, and changes to ovarian follicle proportions. Because the reductions in male organ weights were relatively small an unaccompanied by microscopic changes, the changes to organ weight did not affect the study director decision for the NOAEL for reproduction.

(c) Nemec, MD. (1995) Two generation reproductive toxicity study of the test material (EC 272-234-3) in rats. WIL Research Laboratories, Inc., Study No. WIL-187006. Conducted for Chevron Energy Technology Company.

Study Design
The study was designed to meet the OECD 416 test guideline requirements in place at the time of the study. Parental generations were identified as F0 and F1. The progeny of the F0

animals are identified as the F1 (pups) until selected and identified as F1 for the second generation. The progeny of the F1 animals were identified as the F2 (pups).
Test Substance: EC272-234-3 (6.7wt% TPP)
Test Animals: Sprague-Dawley Crl:DC CD® BR Rats; N = 30/sex/group (F0)
Dose levels: 0 (peanut oil), 50, 300, 1000 mg/kg/day (equivalent to 0, 3.4, 20.1, 67 mg TPP/kg/day)

Dosing period: Daily, at least ten weeks prior to mating for adults (both sexes, both generations), throughout mating, gestation and lactation; F1 offspring selected to form the second parental generation began direct dosing after weaning. To further investigate reproductive findings that occurred in the first generation, a second group of control and high dose animals (F1 Satellite) were retained for a cross-breeding phase of the study (treated males x untreated females; untreated males x treated females).

Summary of No Observed Adverse Effect Levels

End Point/Generation	NOAEL (mg/kg/day)
Parental (F_0, F_1)	50 (3.4 mg TPP/kg/day)
Reproduction (F_0, F_1)	300 (20.1 mg TPP/kg/day)
Offspring (F_1, F_2)	50 (3.4 mg TPP/kg/day)

Summary of Results Consistent with TPP Reproductive Toxicity
(note: all findings consistent with reproductive effects of TPP, whether attributed to test substance exposure or not attributed, are shown below; study director justification included as appropriate. Age at sexual maturation, estrous cyclicity, and semen quality were not evaluated in this study.)
1000 mg/kg/day (67 mg TPP/kg/day)
- decreased ovary weight
o F0: 0.1221 g vs. 0.1508 g (control)
o F1: 0.1532 g vs. 0.1365 g (control); not statistically significant
- decreased pregnancy rate
o F0: 73.3% vs. 96.7% (control)
o F1: 76.7% vs. 93.3% (control)
☐ F1 satellite (treated males x untreated females): 89.3%
☐ F1 satellite (untreated males x treated females): 55.2%
- decreased live litter size
o F0: 8.8 pups (mean) vs. 12.6 pups (control)
o F1: 6.8 pups (mean) vs. 13.0 pups (control)
☐ F1 satellite (treated males x untreated females): 12.6 pups
☐ F1 satellite (untreated males x treated females): 5.8 pups

- decreased epididymal weight in presence of reduced body weight in males (ca. 86% of control)
o F0: 1.35 g vs. 1.47 g (control)
o F1: 1.25 g vs. 1.50 g (control)
300 mg/kg/day (20.1 mg TPP/kg/day)
- decreased epididymal weight in presence of reduced body weight in males (ca. 93% of control)
o F0: no effect
o F1: 1.25 g vs. 1.50 g (control)

Additional Reproductive Findings (note: In studies conducted with TPP, testes weight was decreased only in the presence of significant systemic toxicity in males.)

1000 mg/kg/day (67 mg TPP/kg/day)
- increased incidence of dead pups (presented as number of pups, not litters)

o F0: 19/187 pups born vs. 3/368 pups born (control)
o F1: 15/131 pups born vs. 8/346 pups born (control)
☐ F1satellite (treated males x untreated females): 6/371 pups born
☐ F1 satellite (untreated males x treated females): 9/79 pups born

Additional Reproductive Findings (note: In studies conducted with TPP, testes weight was decreased only in the presence of significant systemic toxicity in males.)

1000 mg/kg/day (67 mg TPP/kg/day)
- increased incidence of dead pups (presented as number of pups, not litters)
o F0: 19/187 pups born vs. 3/368 pups born (control)
o F1: 15/131 pups born vs. 8/346 pups born (control)
☐ F1satellite (treated males x untreated females): 6/371 pups born
☐ F1 satellite (untreated males x treated females): 9/79 pups born
- dystocia
o F0: no occurrences
o F1: 4 females
- decreased testes weights
o F0: no effects
o F1: 3.52 g vs. 3.82 g (controls)
o F1 Satellite: 3.77 g vs. 3.82 g (controls)
- pup growth reduced
o F1: 7 – 11% lower pup body weight than control value, postnatal days 14 – 28
o F2: no effect

300 mg/kg/day (20.1 mg TPP/kg/day)
- increased incidence of dead pups (presented as number of pups, not litters)
o F0: 26/372 pups born vs. 3/368 pups born (control)
o F1: 3/371 pups born vs. 8/346 pups born (control)
- dystocia
o F0: no occurrences
o F1: 1 female

Other General Macropathology Findings
In both groups of F1 generation males, ejaculatory plugs were observed in the cage-pan liners of unpaired males prior to the mating phase of the study, with the highest frequencyof plugs noted in the highest dose group. The biological significance of this finding is not known.

Summary of Parental Toxicity Findings
Males
1000 mg/kg/day (67 mg TPP/kg/day): body weight reduced to approximately 79% - 86% of control (515 g vs. 600 g (F0); 496 g vs. 627 g (F1); increased kidney weight(absolute or relative to body weight) (F0, F1); increased relative liver weight (F0, F1); no microscopic changes attributed to treatment 27

300 mg/kg/day (20.1 mg TPP/kg/day): body weight reduced to approximately 87% - 93% of control (558 g. vs. 600 g (F0); 550 g vs. 627 g, (F1); increased kidney weight (F0); increased relative kidney weights (F0, F1); no microscopic changes attributed to treatment
50 mg/kg/day (3.4 mg TPP/kg/day): no effects

Females
1000 mg/kg/day (67 mg TPP/kg/day): transient reductions in body weight/change (F0, F1)

(note: body weight reductions during gestation were likely due to smaller number of fetuses); increased liver weight (absolute and relative to body weight) (F0, F1); increased relative kidney weights (F1); no microscopic changes attributed to treatment
300 mg/kg/day (20.1 mg TPP/kg/day): transient reductions in body weight/change (F0); increased relative liver weight (F1); no microscopic changes attributed to treatment
50 mg/kg/day (3.4 mg TPP/kg/day): no effects

Conclusions
Adverse effects consistent with TPP reproductive toxicity included reduced ovary weight, reduced pregnancy rate, and reduced litter size at 1000 mg/kg/day (67 mg TPP/kg/day). Cross-mating of treated animals of either sex with untreated partners indicated that the fertility effects observed on pregnancy rate and litter size aligned with treatment of the female rat. Reductions in epididymal weights occurred at 1000 and 300 mg/kg/day (67 and 20.1 mg TPP/kg/day, respectively) in the presence of body weight reductions of similar magnitude. Other endpoints such as estrous cyclicity and acceleration of female sexual maturation were not measured in this study, as this study predates the current OECD test guideline.

In this study, other reproductive effects observed but inconsistent with the profile of TPP reproductive toxicity included increased pituitary weight, particularly in males, dystocia, and dead pups after parturition. Testes weight was reduced in the presence of reduced male body weight (ca. 79% - 86% of control values). Pup growth was also reduced compared to the concurrent control F1 litters but was unaffected in the F2 litters (TPP administration had reduced pup growth, but at levels with more significant effects upon fertility).

(d) Knapp, JF. (2008) An oral (gavage) one-generation reproductive toxicity study of [EC 255-880-2] in rats. WIL Research Laboratories, LLC, Study No. WIL-187045. Conducted for Chevron Energy Technology Company, Toxicology and Health Risk Assessment Unit.

Study Design
The study was designed to meet or exceed the OECD 415 test guideline requirements, with additional parameters (estrous cyclicity, semen analysis, hematology, serum chemistry) included for parental animals. The parental generation was identified as F0. The progeny of the F0 animals were identified as the F1 (pups).
Test Substance: EC 255-880-2 (2.5wt% TPP)
Test Animals: Sprague-Dawley Crl:CD(SD) Rats; N = 30/sex/group (F0)
Dose levels: 0 (corn oil), 50, 170, 500 mg/kg/day (equivalent to 0, 3.4, 20.1, 67 mg TPP/kg/day)
Dosing period: Daily, at least ten weeks prior to mating for adults (both sexes, both generations), throughout mating, gestation and lactation.

Summary of No Observed Adverse Effect Levels

End Point/Generation	NOAEL (mg/kg/day)
Parental (F_0)	170 (4.24 mg TPP/kg/day)
Reproduction (F_0)	500 (12.5 mg TPP/kg/day)
Offspring (F_1)	500 (12.5 mg TPP/kg/day)

Summary of Results Consistent with TPP Reproductive Toxicity
There were no effects upon reproductive parameters associated with TPP reproductive toxicity (no effects upon ovary weight, fertility, litter size, estrous cyclicity, female sexual maturation).

Summary of Parental Toxicity Findings

Males
500 mg/kg/day (12.5 mg TPP/kg/day): increased prothrombin time and activated partial

prothrombin time; reduced red cell count, decreased reticulocyte count; increased alkaline phosphatase; increased liver weight (absolute and relative to body weight); increased kidney weight (absolute and relative to body and brain weights); increased adrenal gland weight (absolute and relative to body and brain weights); increased pituitary weights (absolute and relative to body and brain weight);
170 mg/kg/day (4.25 mg TPP/kg/day): increased prothrombin time and activated partial prothrombin time; decreased reticulocyte count; increased liver weight; no microscopic changes attributed to treatment.
50 mg/kg/day (1.25 mg TPP/kg/day): no effects

Females
500 mg/kg/day (12.5 mg TPP/kg/day): increased alkaline phosphatase; decreased serum cholesterol; no microscopic changes attributed to treatment.
170 mg/kg/day (4.25 mg TPP/kg/day): no effects
50 mg/kg/day (1.25 mg TPP/kg/day): no effects

Conclusions
Adverse effects consistent with TPP reproductive toxicity were not observed in this study, nor were other reproductive effects. Systemic effects were minimal and included evidence of effects upon hematological parameters, serum chemistry, and organ weight changes without microscopic effects. Effects upon adrenal and pituitary gland weights were attributed by the study director as consequences of general physiological stress and not specific to the test substance.

Dossier Submitter's Response

We thank Chevron Oronite SAS for their comments, and for introducing new information that was not available to us when the dossier was prepared. With the exception of the mechanistic studies (see below), responses regarding the findings of the repeated-dose studies, including those on substances that contain an estimated amount of residual TPP as an impurity, can be found in the various responses to Member States Competent Authority comments.
Uterotrophic assay (Edwards, T.L. et al., 2010a).
Chevron Oronite SAS cites a single uterotrophic assay which reported a significant increase in wet and blotted uterine weights over the dosing range from 75 to 500 mg/kg TPP. However these findings are inconsistent with the observed reduction in absolute and relative uterus weights in the female pubertal assay over a range of doses up to 800 mg/kg/day. Female rats treated with 125 mg/kg/day group (high dose) in the 1-generation reproduction toxicity study responded with a statistically increase in uterus weight whereas F0 female rats dosed at 75 mg/kg/day in the 2-generation dietary study were found to have a non-statistically significant reduction in uterine weights. The inconsistency in the effects of TPP on the uterus over these multiple study designs casts significant doubt on the validity of the conclusion that a relevant mechanism of action has been identified.
Female pubertal assay (Knapp, JF. 2009)
Chevron Oronite SAS link the outcome of female pubertal assay parameters to a direct effect of TPP in rats, and thus of potential relevance to humans. A careful review of the female pubertal assay results provides a number of key observations that highlight inconsistencies in the overall data set however, and therefore mitigate the relevance of these data. For example, in the female pubertal assay at doses of 5, 20 and 60 mg/kg/day there was no statistical difference in vaginal opening, no dose-response over the very broad range of doses and no comparison of the observed marginal changes versus controls with laboratory historical control data. With respect to decreased numbers of corpora lutea it is difficult to substantiate the conclusion reached by the commentators because there was no statistical effect and a comparison with laboratory historical controls is missing. Similarly, it is difficult to substantiate the conclusion with regard to uterine hypoplasia because there

was no statistical effect, no dose-response and no comparison with laboratory historical control data. Furthermore the commentator acknowledges that even at very high doses of TPP no changes were observed in circulating E2 and LH levels. If TPP was responsible for producing adverse effects directly on reproductive systems in the rat by direct estrogenic mechanisms (as opposed to secondary effects due to generalised systemic toxicity) then it would be reasonable to expect that there should be evidence of compensatory changes in hypothalamic-pituitary-gonadal axis as manifested by alterations in the circulating LH levels. This was evidently not the case.
Histological assessment of the animals treated with TPP in the female pubertal assay showed decreased corpora lutea at doses that cause systemic toxicity (200 and 800 mg/kg/day) and uterine atrophy at 800 mg/kg, a clear systemically-toxic dose.
Furthermore, the observed effects on the uterus directly contrast with the increased uterine weights at comparable dose levels in the uterotrophic assay. It is therefore difficult to derive any meaningful conclusion from these mechanistic assays, especially as the high doses caused clear systemic toxicity, and so the data have questionable relevance to establishing a plausible mechanism in humans.
Estrous cycle evaluation in the female pubertal assay could only be determined on 5/15 control animals due to high number of females with incomplete cycles which calls into question the validity of the study. Nevertheless, with the remaining animals, no differences in estrus cycle lengths were noted at doses relevant to the repeated dose studies interpretation (i.e., 10, 50 and 200 mg/kg/day groups).

Ligand binding assays
Chevron Oronite SAS suggest that TPP is a weak competitive ligand both for the estrogen receptor and the androgen receptor. In the absence of a negative control (a control substance that has been confirmed not to have affinity for estrogen of androgen receptors), findings in these two experiments should be carefully considered. There is no discussion of the potential for non-specific binding, which could be an alternative explanation for the study results. With regard to the estrogen binding study, there is no explanation or point of comparison for the findings that TPP had the ability to begin competing at 10^{-7} M. Such isolated mechanistic in vitro studies do not allow any definite conclusions regarding the mechanism of action or the potential of TPP to cause adverse effects on the reproductive system in humans. Finally, no evidence is presented that such weak binding activity results in biological or toxicological effects in vivo.

RAC's response

Thank you for provision of additional data and the analysis of existing evidence.

The calculations of the concentration limits in the RAC opinion are based on current ECHA guidance. For medium potency substances, such as TPP, the GCL should be used, namely 0.3 % for substances classified as Repr. 1B according to the CLP Regulation.

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	Germany		MemberState	26

Comment received

The German CA agrees that the existing data for TPP support the classification for Repr. Cat. 2 H361 f (CLP Regulation).

In addition it should be mentioned on p.77/78 2-gen study: Please add a justification why pup effects (Tabel 40) at 15 mg/kg bw/d (dose without maternal toxicity) are considered as not relevant for classification for developmental effects.

On p. 92: It is noted, that the whole section/page for lactation toxicity is copied from CLP.

Short citations and comparison with observed effects is preferred instead of copying whole passages from guidance text.
Dossier Submitter's Response
Thank you for your response. We agree with the position that existing data supports the classification of PDDP as Repr. Cat. 2 (CLP Regulation). The pup effects observed at 15 mg/kg bw/day in the 2-gen study was statistically significant on days 14 and 21 in the F1 offspring. The findings, however, were considered not to be toxicologically-significant because no similar statistically significant effects were observed in the F1A and F1B offspring at this dose level. See also the additional comment (number 25) in respect the dietary concentrations that were not adjusted during the lactation period. As a consequence of the much higher food consumption by maternal rats they received a much higher dose than intended which confounds any interpretation of findings amongst offspring.
RAC's response
The option of classification of TPP as Repr. 2 was considered by RAC; however, classification as Repr. 1B was considered justified based on the information provided to RAC. The rationale and justification for the classification as Repr. 1B is contained in the opinion document. For developmental toxicity, see RAC response to comment No 18.

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	Germany		MemberState	27
Comment received				
p.42: The argumentation that substance inhalation is unlikely due to the low volatilisation point and pattern of use could be repeated here.				
Dossier Submitter's Response				
Thank you for your response. We agree with this comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
29.01.2013	United Kingdom		Company-Downstream user	28
Comment received				
No data available but lack of skin sensitisation potential suggests no cause for concern.				
Dossier Submitter's Response				
Thank you for your response. We agree with this comment.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	France		MemberState	29

Comment received
• Skin irritation: The proposed classification is R38 and not R36 as mentioned.
Dossier Submitter's Response
Thank you for your response. It is indeed proposed to classify the substance in accordance to the DSD as Xi, R36/38 Irritant; Irritating to eyes and skin
RAC's response
Thank you for your comment. In the opinion of RAC, taking into account all studies, it is assumed that the eye effects are reversible, and there is not enough data to warrant classification in Eye Dam. 1, therefore classification Eye Irrit. 2 was considered more appropriate. However, since RAC also concluded on classification as Skin Corr. 1C with hazard statement H314: Causes severe skin burns and eye damage based on skin irritation/corrosion assessment, the classification Eye Irrit. 2 will not be included in Table 3.1, Annex VI to CLP. For further information, see Note 1 to the Classification table in the opinion document.

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Sweden		MemberState	30
Comment received				
The Swedish CA supports the proposed classification of Phenol, dodecyl-, branched / Phenol, (tetrapropenyl) derivatives (CAS nr 121158-58-5, 74499-35-7) as a category 2 skin irritating compound. The mean score for erythema as well as for oedema was >2.3 (studies by Waid et al 1989 and Cavelli et al 1968) and thus classification as a Cat 2 skin irritant is warranted. The effects produced after 4 hours are not of such severity (no ulcers etc.) that a classification in category 1C is warranted. Specific comments Four other studies (out of which three are stated as supportive studies) are reported (p 34). However, clarification of test methology (or a reference to guideline) and/or of the endpoint "primary dermal irritation index" are lacking, and before this has been clarified it is not possible for the reader to properly assess the result from these studies. In Section 4.4.1.4, please specify the reason for classification (mean score ≥2.3 but ≤ 4), as well as the reason for not classifying this compound in Cat 1C.				
Dossier Submitter's Response				
We appreciate your support of the proposed skin irritation classification In the supporting studies, the reliability of two studies (Cavalli *et al.* 1968, Randall & Robinson 1990) are valid with restrictions (Klimisch Code = 2) because the animals were exposed with the test material for 24 hours instead of 4 hours under current testing guidelines. The Cavalli study at 7 days showed that skin was necrotic and lifting; fur-bearing skin was noted below the lifting which is an indication that there was not full skin destruction. Three other studies (Mürmann 1984b, 1988, 1991) were conducted with exposure times of 3 minutes or 4 hours, but the exposed skin was washed with warm water to remove the test material. Due to the low water solubility of dodecylphenol, it is unlikely that washing with water would be an effective means of removing the test material a specified in the current testing guideline for skin irritation, OECD Test Guideline 404. Therefore, these three studies are considered invalid (Klimisch Code = 3).				

Species	Results	Reliability	Reference
Rabbit	24-hr exposure Primary Irritation Score = 8.0/8.0 No in-depth skin changes observed at 7 days	Klimisch Code = 2	Randall & Robinson 1990
Rabbit	4-hr exposure, warm-water wash Primary Irritation Index = 6.09/8.0 Mean Erythema (0-4): 24 hours = 4 48 hours = 4 72 hours = 4 Mean Edema (0-4): 24 hours = 2.7 48 hours = 2.0 72 hours = 2.0 Necrosis present at study termination (Day 6).	Klimisch Code = 3	Mürmann 1984b
Rabbit	3-min exposure, warm water wash w/ one animal Primary Irritation Index = 6.75/8.0 Mean Erythema (0-4): 24 hours = 4 48 hours = 4 72 hours = 4 Mean Edema (0-4): 24 hours = 4 48 hours = 4 72 hours = 4 Necrosis present at study termination (Day 5)	Klimisch Code = 3	Mürmann 1988
Rabbit	3-min exposure, warm water wash w/ one animal Primary Irritation Index = 6.5/8.0 Mean Erythema (0-4): 24 hours = 4 48 hours = 4 72 hours = 4 Mean Edema (0-4): 24 hours = 4 48 hours = 4 72 hours = 4 At study termination (Day 17), all skin injuries had healed with scar formation	Klimisch Code = 3	Mürmann 1991

Conclusions on classification and labelling

Based on the key study that did not show necrotic changes, Phenol, dodecyl-, branched is classified for skin irritation (Category 2): H315-Causes skin irritation. Classification as a skin irritant (R38: irritating to skin) is required according to the criteria of the Dangerous Substances Directive.

RAC's response

In the analysis of skin irritation/corrosion RAC has taken into account the following studies:

- The scores and skin observations in the Waid *et al.* study (1989) indicating that TPP

induced very severe skin inflammation lasting until the end of 14 days after 4-hour dermal exposure with signs of skin necrosis in some rabbits, but not confirmed in histopatological examinations. The 14-day observational 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 because after 4 hours of rabbit skin exposure (number of animals tested not known) the skin was washed with warm water to remove the test material, which could not be sufficient to remove the substance due to its low water solubility.
- In Cavalli *et al.* (1968), 7 days after 24-hour dermal exposure, strong skin inflammation was observed and skin was reported to be necrotic in some rabbits.
- Two studies in rabbits with dermal exposure reported to last only 3 minutes (it may have lasted longer due to insufficient removal of the substance from the skin). Signs of necrosis was reported on 5th day after exposure in one study (Mürmann, 1988) and in a 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 the studies indicate that TPP causes skin necrosis in rabbits; however, due to poor description and uncertain duration of exposure in some studies it is not possible to precisely determine the time of exposure leading to these effects. Using a weight of evidence approach with all available studies it is conclude that TPP should be classified according to CLP legislation as Corrosive in subcategory 1C (for substances where responses occur after exposures between 1 hour and 4 hours and observations up to 14 days) with the hazard statement H314: Causes severe skin burns and eye damage.

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	Belgium		MemberState	31
Comment received				
• For the skin irritation, all the data indicate an irritation (Waid et al's study (1989)): ☐ erythema score is 4/4 at 24, 48 and 72h and not fully reversible within 14days. ☐ oedema score is 3,4/4 at 24h, 2,6 at 48h and 2,8 at 72h. Thus the criteria for categorie 2, mean values for erythema and/or oedema in min 2/3 animals: >2,3 and <4,0 and inflammation continue at the end of the observation time (14d) in min 2 animals are fulfilled.				
Dossier Submitter's Response				
See comments provided above on this endpoint (comment 30).				
RAC's response				
See RAC response to comment No 30.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	France		MemberState	32
Comment received				
• Eye irritation: some information is lacking: dose, period of exposure and observation, rinse and number of animals.				

No information on reversibility after 72h is available. In this context, it is difficult to conclude on classification H318 or H319 (R36 or R41). The proposed classification as an eye irritant is R36 or R41 and not R38 as mentioned.

Dossier Submitter's Response

Summary of Primary Eye Irritation Data

Species / Method	Results	Reliability	Reference
Rabbit equivalent or similar to OECD Guideline 405	24-hr Draize score = 5.0/110 No corneal opacity or iritis Mean Conjunctival Redness: 24 hours = 2.0 48 hours = 1.7 72 hours = 1.3 clear of irritation by Day 10	Klimisch Code = 1	Waid *et al.* 1990
Rabbit / Method equivalent or similar to OECD Guideline 405	24-hr Draize score = cannot be calculated ; area of corneal opacity was not recorded Mean Conjunctival Redness: 24 hours = 3.0 48 hours = 2.7 72 hours = 2.3 Terminated at 72 hours, extent of recovery not determined	Klimisch Code = 4	Cavalli *et al.* 1968
Rabbit / Method-Federal Hazardous Substances Act, 21 CFR, § 191.12 (1964)	24-hr Draize score = 33.3/110 no mention if all eyes were clear of irritation at the end of the study	Klimisch Code = 2	Randall & Robinson 1990
Rabbit 0.1 mL of the test material (97% purity) was applied to the eyes of six rabbits. They were observed for up to 21 days.	24-hr Draize score = 19.3/110 Corneal opacity & iritis in 5/6 animals; persisting in 1 animal through 21 days Mean Conjunctival Redness: 24 hours = 2.5 48 hours = 2.2 72 hours = 2.0	Klimisch Code = 2	Mürmann 1984c

Conclusions on classification and labelling

The ocular responses observed in various studies require classification of the substance for serious eye irritation (Category 2) H319-Causes serious eye irritation under CLP. Classification as an eye irritant (R36: irritating to eyes) is required according to the criteria of the Dangerous Substances Directive.

RAC's response

See RAC response to comments No. 6 and No. 30.

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Sweden		MemberState	33

Comment received

The Swedish CA supports the proposed classification of Phenol, dodecyl-, branched / Phenol, (tetrapropenyl) derivatives (CAS nr 121158-58-5, 74499-35-7) as a category 2 regarding eye damage/eye irritation. Two OECD guideline 405 studies are reported with contradictory results. In Waid et al 1990, mean readings for cornea score, iris score, conjunctivae score

and chemosis score are all below the range that warrants a classification. In addition, this study indicates reversibility of the signs within 10 days. In contrast, the study by Cavelli 1968 reports a mean reading above 2 (from the 24, 48 and 72 hours readings) for both the conjunctivae score and the chemosis score. This information in itself justifies a classification of the substance as a category 2 eye irritating compound. The result from the Cavalli et al study regarding corneal opacities or iritis is not of such severity that it justifies a classification in category 1. Specific comments Page 37: Two other studies are reported (Randell 1978 and Anon 2006). For the Randell study, more information regarding how to interpret the endpoint ”mean overall irritation score” is needed in order to evaluate this study. Also for the Anon study, the endpoints ”mean overall irritation score” is used as is the endpoint ”mean overall score ” without any explanation (and no info on this endpoint is provided in the CLP guideline). Please include this information so that the value of these studies can be assessed. It is however of interest to note that also in the study by Anon et al a mean conjunctivae score >2 was recorded, i.e. data which support a category 2 classification. In Section 4.4.2.3, please add that the severity of the finding only exceeded classification threshold for category 2 but not for category 1. In Section 4.4.2.4, please add in text that provides details why the compound should be classified in category 2.
Dossier Submitter’s Response
We appreciate your support for the proposed eye irritation classification. See additional study comments provided above on this endpoint (comment 32).
RAC’s response
See RAC response to comments No. 6 and 30.

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	Belgium		MemberState	34
Comment received				
• For the eye irritation, (Cavalli et al’s study) ☐ the cornea score is 1,7/4 at 24h, 0,7/3 at 48h and not fully reversible within 72h, ☐ the conjunctival score is 3/3 at 24h, 2,7/3 at 48h, 2,3/3 at 72h and not fully reversible within 72h, ☐ the chemosis score is 3,3 at 24h, 3,5 at 48h, 3 /4 at 72h and not fully reversible within 72h. Thus, the criteria for categorie 2 at least in 2 of 3 animals, a positive response of corneal opacity >1, iritis >1, conjunctival redness >2, conjunctival oedema >2 are fulfilled.				
Dossier Submitter’s Response				
We appreciate your support for the proposed eye irritation classification.				
RAC’s response				
See RAC response to comments No. 6 and 30.				

OTHER HAZARDS AND ENDPOINTS – Skin Senzitation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	France		MemberState	35
Comment received				
• Sensitisation: some information is lacking: choice of doses (irritation or not), period of exposure, number of application. Moreover, the percent of positive response is superior or equal to 15% in the 2 studies available. In this context it is not possible to conclude on the				

sensitizing potential of the compound.

Dossier Submitter's Response

In the Morris study, the potential of the test material as a 2.5% w/v in Mineral Oil to produce delayed contact hypersensitivity in guinea pigs was evaluated. Following primary challenge using the test material, as a 1% w/v formulation in Mineral Oil, the incidence of grade 1 responses in the test group (2 of 19) was compared to that of the naive control group (1 of 10). The incidence and severity of these responses in the test group were essentially comparable to those produced by the naive control group suggesting that sensitization had not been induced. However one test animal responded with a grade ± at the 24 hour reading which increased to a grade 1 at the 48 hour reading. This type of response is suspect as a sensitization type response. Therefore, a rechallenge was conducted to clarify the response noted during primary challenge.
Following re-challenge using the test material, as a 1% w/v formulation in Mineral Oil, the incidence of grade 1 responses in the test group (5 of 19) was compared to that of the naive control group (2 of 10). The incidence and severity of these responses in the test group were again essentially comparable to those produced by the naive control group. The failure of the test animals to exhibit a higher incidence of responses over that of the naive control group indicates that the responses noted are due to irritation and not sensitization.

In the Silveira study, the skin sensitization potential of 5% of the test material (w/w in mineral oil) was determined using the modified Buehler test. Each compound was tested on a group of 15 male guinea pigs. The vehicle, U.S.P. mineral oil and the positive control material, DNCB, were tested concurrently with each test material on groups of ten male guinea pigs.
Skin Irritation During Induction Period:
Initial application of 5% of the test material (w/w in mineral oil) produced very slight erythema in three of 15 animals and well-defined erythema which decreased to very slight by 48 hours in one of 15 animals. Repeated topical administration, as evaluated 24 hours after the fifth application, resulted in very slight to moderate erythema and no to slight edema for all 15 animals. Thirteen of 15 animals showed very slight to well-defined erythema and no to very slight edema 24 hours after the tenth application.
Response to Challenge:
None of the 15 animals induced and challenged with 5% of the test material (w/w in mineral oil) showed sensitization reactions. Very slight erythema was observed in three animals 48 hours after challenge. Initial and challenge scores were not statistically different.

RAC's response

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. Skin sensitisation was not proposed for harmonized classification by the Dossier submitter and no data were provided in the CLH report; therefore this hazard class was not considered.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	Germany		MemberState	36

Comment received

p.32: Clarification to which study/studies section 4.3.1 is referring is suggested.

Dossier Submitter's Response

According to the criteria of the CLP (Regulation 1272/2008/EC), Specific target organ toxicity (single exposure) is defined as specific, non lethal target organ toxicity arising from a single exposure to a substance or mixture (except acute toxicity, skin corrosion/irritation,

serious eye damage/eye irritation, skin and respiratory sensitization, CMR properties, aspiration).
In the acute oral toxicity study, mortality was observed from 1260 mg/kg bw onwards. The observed clinical findings were described ruffled fur and later on a crouched posture, diarrhea, diuresis, bloodstained nose, deep red eyes, retarded motion, slight to medium sedation and ataxia. At the high dose the animals occasionally assumed a prone position. All surviving animals were free of signs of toxicity after 10 days. The effects are considered to be secondary due to the high, acutely toxic dosages leading also to mortality. The effects observed in the acute toxicity study after single oral exposure to high dosages which lead also to mortality do not require a classification with regard to STOT SE.

There were no findings in any of the other acute toxicity studies or from human experience considered relevant for a STOT (SE) classification.

RAC's response

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. No justification and no data were provided in the CLH report; therefore this hazard class was not considered.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	Germany		MemberState	37

Comment received

p. 43/44
Please indicate dose-dependency of thyroid gland hypertrophy at 5 mg/kg and higher doses.

p. 44/45
More details should be mentioned in the Table 18 on the nature of effects on the reproductive tract observed in the study of Vogin (1970a).

p. 47 and p. 59 (Reyna & Thake, 1988): Please correct the headings of Table 27 and 28. Both refer to the diet study (instead of gavage). Table 28 reports bone marrow hyperplasia at 300 mg/kg bw/d. This effect is a serious effect at the guidance value for classification with STOT RE. The information lacks in section 4.7. (Table 18 and p. 47/48). Effects on hematology parameters should be reported, if available (or whether examined). Based on the depressed growth and the conclusion that reduced food consumption was not primarily associated to unpalatability of the diet the NOAEL should be 40 mg/kg bw/d.

p. 48 and p. 61/62 (Harriman, 2004) Anaemic effects at doses relevant for classification are reported on p. 61, but lack in the summary on p. 48. The level of reduction should be given for relevant parameters to allow decision on STOT RE.

Liver cell vacuolation is an adverse effects that may be relevant for classification at the doses observed (≥180 mg/kg bw/d, 28-day study).
Severity scores should be added to allow conclusion on the necessity of classification.

p. 48/49 (Haas, 2007) More data on severity or amount of reduction are also needed for the anaemic effects and liver cell vacuolation that were observed in the 90-day study.

p.50: It is noted that in 4.8.2. a comparison of observed adverse effects with the criteria should take place, but only the CLP regulation is cited.

p. 51 Systemic or organ-specific toxic effects occurring at oral doses up to 300 mg/kg bw/d in a 28 day study may be relevant for classification (see Table 3.9.2.2. Guidance to CLP regulation). The same is true for doses up to 100 mg/kg bw/d which is the guidance value for 90-day studies.

It is recommended to compare the observed non-specific and organ-specific effects to the CLP guidance values.

Dossier Submitter's Response

P43/44
28-Day Oral (Gavage) Study in Rats (Harriman 2004) dose response of thyroid microscopic findings

Parameter	**Dose Level (mg/kg/day)**					
	0	**5**	**20**	**60**	**180**	**300**
Microscopic Findings (incidence)						
• Thyroid – follicular cell hypertrophy, M	0/5	1/5 (1)	1/5 (1)	2/5 (1)	3/5 (1)	3/5 (2-3)
• Thyroid – follicular cell hypertrophy, F	0/5	NE	NE	0/5	0/5	0/5
NE = Not examined; ( ) = severity of microscopic changes: 1 = minimal; 2 = mild; 3 = moderate; 4 = severe						

p44/45
Findings of relevance to reproductive toxicity

Mean absolute and relative testes weights were reduced in males dosed at 200 mg/kg bw/d with testicular hypospermia observed in six out of 20 animals in the Vogin study (1970a). Additionally, liver weights were increased among either sex dosed at 200 mg/kg bw/d. No additional histopathological effects attributable to treatment with the test substance were noted in this study.

p. 47 and p. 59 (Reyna & Thake, 1988):
The Reyna, M.S., and Thake, D.C. 1988 is indeed a Four week Feeding Study of Dodecylphenol in Sprague-Dawley Rats.
Bone marrow hypoplasia of minimal severity were present in males (6/10) and females (3/10) at the high dose level (5000 ppm corresponding to 250 mg/kg bw) but not at the next lower level (2500 ppm corresponding to 125 mg/kg bw). Such effects were not seen in the 90-d repeat dose toxicity studies.
At study termination, mean reticulocyte counts were statistically significantly reduced only in the 2500 and 5000 ppm males (by 44% and 65%) compared to the controls. In addition, mean haematocrit values were statistically significantly lower in the 5000 ppm males (by 5%) and females (by 6%), and a significant increase (by 6%) in mean corpuscular haemoglobin concentration was observed in the 5000 ppm males. The LOEL for effects on red blood cell parameters was 2500 ppm but associated with significant body weight depression (-33%).
p. 48 and p. 61/62

Parameter	Dose Level (mg/kg/day)					
	0	5	20	60	180	300
Mean Haemoglobin (g/dL), M	14.8	15.4	14.6	15.2	14.2	14.3
Mean Haemoglobin (g/dL), F	15.3	14.3	14.5	14.9	14.0**	13.5**
*p < 0.05; **p < 0.01						

The above effects are considered relevant for the assessment of the STOT-RE classification.

As these effects were either not reproduced in the 90 day repeat dose toxicity studies or at doses above the guidance threshold, these findings were considered not sufficient to justify classification as STOT-RE.
RAC's response
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. No justification were provided in the CLH report; therefore this hazard class was not considered. However, the information on repated dose toxicity was considered, where relevant, in the discussion on reproductive toxicity.

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	France		MemberState	38
Comment received				
• Repeated toxicity: there are some inconsistencies between the text and the table (for example: Vogin's study (1970a) with a NOEL of 25 mg/kg/d in the table whereas effects were observed at 200 mg/kg/d). In the study of Hass, as some effects on epididymes, prostate and seminal vesicles were observed at 100 mg/kg/d, the NOAEL for reproductive tract effect should be 50 mg/kg/d and not 100, as mentioned. The number of animals should be mentioned for all studies.				
Dossier Submitter's Response				
In the Vogin (1970a) study (20 animals per dose group and sex) the only effects observed at 100 mg/kg/d was a mean body weight gain reduction in females by 8%. These effects have not been mentioned in the study table (Table 18). In the Haas 90-day repeated dose toxicity study dodecylphenol was administered by to 5 groups (Groups 1-5) of Crl:CD(SD) rats at dose levels of 0, 50, 100, 150 and 200 mg/kg/day. Each group consisted of 10 animals/sex. Organ weight effects on epididymes, prostate and seminal vesicles were observed at 100 mg/kg/d. These were not associated with histological changes. The study director considered the effects at 100 mg/kg/day not to be adverse and therefore assigned the NOAEL at 100 mg/kg/day.				
RAC's response				
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. No justification were provided in the CLH report; therefore this hazard class was not considered. However, the information on repeated dose toxicity was considered, where relevant, in the discussion on reproductive toxicity.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	United Kingdom		MemberState	39
Comment received				
We disagree with the M factor proposed for H400, as classification has not been derived using the most sensitive acute endpoint. We propose M=10, based on the acute Daphnia EC50 = 0.037mg/l. This also aligns with the previous UK classification proposal for the environment agreed at the Meeting on Environmental Effects of Existing Chemicals, Pesticides & New Chemicals on 26th January 2007 (ref. ECBI/131/06 Rev.1. - UK proposal; ECBI/08/07 - meeting summary record).				

Two small editorial points:
Section 5.2.1 - Adsorption/desorption
There is a typo in table 51 as the quoted Koc (10-4 as opposed to 10+4). This has no effect on the classification but could cause confusion.

Section 5.4.1.1 - Short-term toxicity to fish.
The key study uses WAFs so the reported result should be quoted as a loading rate rather than concentration - i.e. LL50 not a LC50 value

Dossier Submitter's Response

For acute toxicity, the Daphnia 48-hour EC50=0.037 mg/L (Sewell & McKenzie, 2005) is considered the most sensitive species. The long-term NOEC (21 d) in Daphnia is 0.0037 mg/L (Sewell & McKenzie, 2005). The M-factor is 10 for acute and chronic classifications according to the criteria specified in the CLP section 4.1.3.5.5.5.1.
Thanks for the additional editorial points.

RAC's response

In the opinion of RAC, 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. Phenol, dodecyl-, branched is therefore classifiable 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-d 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) so is not relevant.

In summary, Phenol, dodecyl-, branched is therefore classifiable as Aquatic Chronic 1 (H400), with an M-factor of 10.

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	Germany		MemberState	40

Comment received

(page 107f.)
The German CA does not agree with the acute M-factor.
Based on the available data the most sensitive species for acute classification is not freshwater algae, but Daphnia magna with EC50 =0.037 mg/L (Sewell & McKenzie, 2005a).
The M-factor (acute) should be 10 instead of 1.
We agree with the proposed chronic classification and the chronic M-factor.

Dossier Submitter's Response

Please see the comments above on the acute classification (comment 39).
We appreciate your support of the chronic classification.

RAC's response

See RAC response to comment No. 39.

Date	Country	Organisation	Type of Organisation	Comment number
30.01.2013	Netherlands		MemberState	41
Comment received				

Biodegradation
Page 98-99: The results of the ready biodegradability screening study show that the substance is not readily biodegradable. This result can be supported although in general, ready biodegradability screening tests have limited meaning for UVCB substances as the test cannot tell whether all constituents are equally degraded. We note the negative inherent study which supports the negative results from the ready biodegradability screening test.

Aquatic Bioaccumulation
Page 100-101: It is not clear if the reported BCF values are lipid normalized. If the reported BCF values are not lipid normalized, lipid normalized values should be provided.

Interpretation and discussion of the BCF test results is missing.

BCF values of 823 and 749 were obtained for 1.1 and 11.1 µg/L (respectively) treatment groups. Based on the information we conclude phenol, dodecyl-, branched, meets the CLP criteria BCF > 500 for bioaccumulation.

Aquatic toxicity
Pages 98-105: None of the robust study summaries for the aquatic toxicity studies say whether the test concentrations where within 20% of nominal throughout the study, but all results are based on nominal concentrations. Please confirm that the concentrations of the substance were maintained with the accepted threshold of at least 80 per cent of the nominal concentration throughout test for the key studies.

Page: 20: In the report from the Environmental Agency referenced within section 1.1 of part B in the CLH report, the results from some of the key studies used in this CLH report have been recalculated based on measured concentrations. Information on the test concentrations in the studies needs to be added and the reported recalculations should be taken into account for the key studies for C&L.

We agree with the proposed classification Aquatic Acute 1 and Aquatic Chronic 1. However, we do not agree with the proposed M-factor for acute toxicity. We propose that the M-factor for acute toxicity should be 10 based on the lowest value is 0.037 mg/l for invertebrate (Daphnia magna) and the substance is not rapidly degradable (also BCF > 500).

Data set Fish Invertebrate Algae Most stringent
Classification Remarks
Acute (LC50, mg/L) 40 0.037 0.36 Aquatic Acute 1; M=10 Based on acute invertebrate results
Chronic (NOEC, mg/L) NA 0.0037 0.07 Aquatic Chronic 1: M=10 Based on available chronic results
Surrogate Aquatic Chronic 3 NA NA Aquatic Chronic 3 Based on surrogate results. Substance is not rapid degradable and BCF > 500
Conclusion on acute aquatic toxicity classification Acute 1; M=10
Conclusion on chronic aquatic toxicity classification Chronic 1, M=10
• Aquatic acute toxicity data are available for all trophic levels. The lowest value is 0.037 mg/l for invertebrates. Classification is Aquatic Acute 1, H400, M=10

• Substance is not rapid degradable and BCF> 500. Chronic data is available for algae and aquatic invertebrates but not for fish. • Based on surrogate data, classification is Aquatic chronic 3 • Based on the most stringent outcome long term classification results in Chronic 1 (M factor 10). Proposal for concentrations limits under DSD should be made based on an LC50 value of 0.037 mg/L Other concerns Page 22: Phenol, nonyl-, branched (CAS 90481-04-2) is listed as one of the substance constituents in Table 6 (section 1.2, Part B) of the CLH report. A typical concentration of 0.6% (w/w) is reported with a concentration range ≥ 0.5% to≤ 4.7%. Evidence from the literature reports the effects of nonylphenol as a clear a disruptor of the sex-hormone balance in fish. Have you taken into consideration the aquatic toxicity effects of nonylphenol in your report? Section 5.2.1 Adsorption/Desorption Page 99: There is inconsistency in the reported Koc/log Koc values in the table and in the text, please clarify what the correct value is.
Dossier Submitter's Response
Aquatic bioaccumulation: The values presented in the report are not lipid normalized. Lipid normalized BCF values were not determined in the study but fish were sampled on day 15 of depuration for lipid content. Calculation of lipid normalised to 5% lipid content BCF values based on this are as follows: Lipid normalized steady-state BCF values were 279, 724 in edible, non-edible fish tissue, respectively, for the 1.1 µg/L treatment group, and 325 and 601 in edible, non-edible fish tissue, respectively, for the 11 µg/L treatment group. Based on the information, dodecyl-, branched, meets the CLP criteria BCF > 500 for bioaccumulation. Aquatic toxicity: Please see the comments above on the acute classification (comment 39). We appreciate your support of the classification. Nonylphenol: Nonylphenol, as based on the REACH dissemination webpage information, shows acute EC50, 48 hr and chronic NOEC, 21d in daphnia magna in a similar order of magnitude as dodecylphenol. Hence, it can be largely excluded that toxicity is influenced by Nonylphenol contained at low concentrations of 0.5-2% in dodecylphenol Section 5.2.1 Adsorption/Desorption The correct adsorption coefficient (Koc) in Table 51 are logKoc: log10 Koc: 1.02 to 4.67
RAC's response
Thank you for the comment. See RAC response to comment No. 17.

Date	Country	Organisation	Type of Organisation	Comment number
29.01.2013	Finland		MemberState	42
Comment received				
In the CLH report the proposed M-factor of 1 for Aquatic Acute Category 1 is based on the				

study with the algae Scenedesmus subspicatus (72h ErC50 = 0.36 mg/l). However, there is also another acute toxicity study available in the CLH report, namely a study on Daphnia magna with a lower EC50 value of 0.037 mg/l. This information would result in an M-factor of 10 for short term hazard and more stringent specific concentrations limits according to Directive 67/548/EEC. We would like to know the dossier submitter's reasoning for not choosing the lowest available EC50 value for setting the M-factor?
Dossier Submitter's Response
Please see the comments above on the acute classification (comment 39).
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Sweden		MemberState	43
Comment received				
The Swedish CA supports the environmental classification of Phenol, dodecyl-, branched / Phenol, (tetrapropenyl) derivatives with CAS nr 121158-58-5, 74499-35-7 as specified in the proposal. SE agrees with the rationale for classification into proposed hazard classes and differentiations. However the dossier submitter has given the wrong name of the algae species considering the chronic toxicity data. Instead of the algae Pseudokircheneriella subcapitata it should be Scenedesmus subspicatus (new name Desmodesmus subspicatus). Phenol, dodecyl-, branched / Phenol, (tetrapropenyl) derivatives fulfills the criteria for classification as aquatic environmental hazard acute category 1, H400 and aquatic environmental hazard chronic category 1: H410. Acute toxicity data are available for all throphic leves. Based on the acute and chronic toxicity data for Scenedesmus subspicatus (new name Desmodesmus subspicatus) (ErC50=0.36 mg/l) and Daphnia magna reproduction (0.0037 mg/L) and the M factors are 1 and 10 for acute and chronic classification respectively, according to the criteria specified in the 2 nd ATP.				
Dossier Submitter's Response				
Please see the comments above on the acute classification (comment 39).				
RAC's response				
Thank you for your comments. They were considered in the justification of the classification.				

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Belgium		MemberState	44
Comment received				
Based on the results of the aquatic toxicity tests and the fact that the substance is not rapidly degradable, it is justified to classify, following the classification criteria of the 2nd ATP, as Aquatic acute, H400 and Aquatic chronic 1, H410. But in our point of view Daphnia magna is the most sensitive species for acute toxicity with a 48hEC50= 0.037 mg/l instead of Scenedesmus subspicatus with a 72hErC50= 0.36 mg/l. This results in an acute M-factor of 10 instead of 1 as the toxicity band for acute toxicity is between 0.01 mg/l and 0.1 mg/l. Chronic toxicity : As there are no chronic data for all 3 trophic levels available, both classifications, based on the NOEC of the available chronic test and the LC50s of the other trophic levels resp., should be assessed and the substance should be classified according to the most stringent outcome. Here, this results in the same classification : Aquatic Chronic				

H410. We agree with the proposed chronic M-factor of 10. Based on the classification and labelling criteria in accordance with dir. 67/548/EEC, 48hLC50 Daphnia magna = 0.037 mg/l, BCF>100, TTP should be classified as N, R50/53 With SCL : N; R50-53: Cn ≥ 2.5 % N; R51-53: 0.25% ≤ Cn < 2.5% R52-53: 0.025 % ≤ Cn < 0.25 % In conclusion : we support the proposed environmental classification, but do not agree with the proposed acute M-factor and SCLs.
Dossier Submitter's Response
Please see the comments above on the acute classification (comment 39). We appreciate your support of the chronic classification.
RAC's response
Thank you for comment.

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Spain		MemberState	45
Comment received				
We are not in agreement with the environmental classification acute categories proposal made, because it was based on the algae EC50 (0.36 mg/L) that we consider it is not the most sensitive specie since Daphnia shows the lower acute toxicity value with a LC50 (48h) equal to 0.037 mg/L (Sewell & McKenzie, 2005a), this study was considered as key study by the raporteur, therefore according to the Daphnia LC50 (48h) and reproduction NOEC (21d) values our environmental classification proposal is: N R 50/53 M=10 according to Directive 67/548/EEC and, Acute 1 M=10 and Chronic 1 M=10, according to CLP Regulation				
Dossier Submitter's Response				
Please see the comments above on the acute environmental classification (comment 39). We appreciate your support of the chronic environmental classification.				
RAC's response				
See RAC response to comment No. 39.				

ATTACHMENTS RECEIVED:

Comments on Annex XV dossiers proposing harmonised Classification & Labelling (File name: COM_CLH_PC_Phenol dodecyl branched_SE.docx), submitted on 01/02/2013 by Sweden MS (*ECHA's comment: additional information provided in the document copied under Toxicity to Reproduction*).

Comments to: CLH Report Proposal for Harmonized Classification and Labeling
Substance Name: Phenol, dodecyl-, branched (abbreviated TPP) (File name: COSAS Comments 31Jan2013 – Final.pdf), submitted on 01/02/2013 by Chevron Oronite SAS (*ECHA's comment: comment copied in the table above)*

Comments on the proposed classification of Phenol, dodecyl-, branched (File name: NL_comment Phenol dodecyl branched 290113_environment.doc), submitted on 30/01/2013 by Netherlands MS