Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community level of

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

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

CLH-O-0000003405-79-03/A1

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

5 December 2013

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name:

EC Number: Phenol, dodecyl-, branched

CAS Number: 310-154-3

Index Number: 121158-58-5

Contact details for dossier submitter: Chevron Oronite SAS, Levallois Perret, France

Version number: 2 **Date: 14 February 2013**

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

The substance phenol, dodecyl-, branched is also known as tetrapropenylphenol or "TPP".

Table 1: Substance identity

Substance name:	Phenol, dodecyl-, branched
EC number:	310-154-3
CAS number:	121158-58-5
Annex VI Index number:	Not listed in Annex VI
Degree of purity:	100%
Impurities:	Not applicable

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Not listed	Not listed
Current proposal for consideration by RAC	Repr. 1B; H360f:May damage fertility [C > 1.5%]	Repr. Cat 2; R60 May impair fertility [C > 1.5%]
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Repr. 1B; H360f:May damage fertility [C > 1.5%]	Repr. Cat 2; R60 May impair fertility [C > 1.5%]

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

The proposed harmonized classification according to the CLP regulation is reproductive toxicity and indicates a need to classify the substance with the hazard statement H360f. Other endpoints are not proposed for harmonized classification and are not discussed in this CLH proposal.

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification	Reason for no classification
2.1.	Explosives	None	Not applicable	Not classified	Conclusive but not sufficient for classification
2.2.	Flammable gases	None	Not applicable	Not classified	Data lacking
2.3.	Flammable aerosols	None	Not applicable	Not classified	Data lacking
2.4.	Oxidising gases	None	Not applicable	Not classified	Data lacking
2.5.	Gases under pressure	None	Not applicable	Not classified	Data lacking
2.6.	Flammable liquids	None	Not applicable	Not classified	Conclusive but not sufficient for classification
2.7.	Flammable solids	None	Not applicable	Not classified	Data lacking
2.8.	Self-reactive substances and mixtures	None	Not applicable	Not classified	Data lacking
2.9.	Pyrophoric liquids	None	Not applicable	Not classified	Data lacking
2.10.	Pyrophoric solids	None	Not applicable	Not classified	Data lacking
2.11.	Self-heating substances and mixtures	None	Not applicable	Not classified	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	None	Not applicable	Not classified	Data lacking
2.13.	Oxidising liquids	None	Not applicable	Not classified	Conclusive but not sufficient for classification
2.14.	Oxidising solids	None	Not applicable	Not classified	Data lacking
2.15.	Organic peroxides	None	Not applicable	Not classified	Data lacking
2.16.	Substance and mixtures corrosive to metals	None	Not applicable	Not classified	Data lacking
3.1.	Acute toxicity - oral	None	Not applicable	Not classified	Conclusive but not sufficient for classification
	Acute toxicity - dermal	None	Not applicable	Not classified	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	None	Not applicable	Not classified	Data lacking
3.2.	Skin corrosion / irritation	None	Not applicable	Not applicable	Not proposed in this CLH request
3.3.	Serious eye damage / eye irritation	None	Not applicable	Not applicable	Not proposed in this CLH request
3.4.	Respiratory sensitisation	None	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	None	Not applicable	Not classified	Conclusive but not sufficient for classification

3.5.	Germ cell mutagenicity	None	Not applicable	Not classified	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	None	Not applicable	Not classified	Data lacking
3.7.	Reproductive toxicity	Repr. 1B	Repr. 1B; H360f: C > 1.5%	Not classified	
3.8.	Specific target organ toxicity –single exposure	None	Not applicable	Not classified	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	None	Not applicable	Not classified	Conclusive but not sufficient for classification
3.10.	Aspiration hazard	None	Not applicable	Not classified	Conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	None	Not applicable	Not applicable	Not proposed in this CLH request
5.1.	Hazardous to the ozone layer	None	Not applicable	Not classified	Data lacking

Labelling:

Signal word: Danger

Hazard pictogram: GHS08: health hazard

Hazard statements: H360f: May damage fertility.

Precautionary statements:
P201: Obtain special instructions before use.

P202: Do not handle until all safety precautions have been read and understood.

P308+P313: IF exposed or concerned: Get medical advice/attention.

P405: Store locked up.

P501: Dispose of contents/container to...

Proposed notes assigned to an entry:

None.

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification	Reason for no classification
Explosiveness	None	Not applicable	Not classified	Conclusive but not sufficient for classification
Oxidising properties	None	Not applicable	Not classified	Conclusive but not sufficient for classification
Flammability	None	Not applicable	Not classified	Conclusive but not sufficient for classification
Thermal stability	None	Not applicable	Not classified	Conclusive but not sufficient for classification
Acute toxicity	None	Not applicable	Not classified	Conclusive but not sufficient for classification
Acute toxicity – irreversible damage after single exposure	None	Not applicable	Not classified	Conclusive but not sufficient for classification
Repeated dose toxicity	None	Not applicable	Not classified	Conclusive but not sufficient for classification
Irritation / Corrosion	None	Not applicable	Not applicable	Not proposed in this CLH request
Sensitisation	None	Not applicable	Not classified	Conclusive but not sufficient for classification
Carcinogenicity	None	Not applicable	Not classified	Data lacking
Mutagenicity – Genetic toxicity	None	Not applicable	Not classified	Conclusive but not sufficient for classification
Toxicity to reproduction – fertility	Repr. Cat. 2; R60; May impair fertility	Repr. Cat 2, R60: C > 1.5%	Not applicable	
Toxicity to reproduction – development	None	Not applicable	Not classified	Conclusive but not sufficient for classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation	None	Not applicable	Not classified	Data lacking
Environment	None	Not applicable	Not applicable	Not proposed in this CLH request

Labelling:

Indication of danger:
T - toxic

R-phrases:
R60 - may impair fertility

S-phrases:
S36/37- wear suitable protective clothing and gloves

S45 - in case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

S53 - avoid exposure - obtain special instructions before use

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

The substance phenol, dodecyl-, branched (also known as tetrapropenylphenol or "TPP") is not listed on Annex VI of the CLP Regulation (EC) No. 1272/2008. The reproductive toxicity classification of TPP does not have consensus among all manufacturers and users. This CLH proposal requests a harmonized classification and specific concentration limit (SCL) for reproductive toxicity. The SCL was determined from existing reproductive toxicity data with TPP and validated against data from existing reproduction studies conducted with TPP-derived substances that contain TPP as an impurity.

2.2 Short summary of the scientific justification for the CLH proposal

The proposal for the harmonized classification of TPP is for reproductive toxicity. Other endpoints do not indicate disharmony among the manufacturers and users. The proposed classification is based on multiple test results that indicate reproducible and consistent adverse effects of TPP on the reproductive system in rats with the potential for relevance of these effects to humans. The weight of evidence from these findings supports a classification for TPP as Category 1B (CLP) or Category 2: R60 (DSD).

Experimental findings from animal studies were evaluated pursuant to the classification criteria in the CLP and DSD for reproductive toxicity. The CLP and the DSD provide general criteria for the classification of reproductive effects. To assist in the development of classifications, guidance from multiple authoritative bodies was reviewed regarding the interpretation of data used for classifying substances for reproductive and developmental toxicity (ECETOC, 2002; ECHA, 2008, 2009; US EPA, 1996; WHO, 2001). According to these documents, reproductive effects are considered relevant for classification if the effects do not appear as secondary consequences to excessive systemic toxicity. Additionally, these guidance documents concur that the total weight of evidence, including reproduction studies, mechanistic assays, and repeat-exposure studies with relevant and reliable information about the reproductive system should be collectively considered.

Exposure to TPP resulted in adverse effects on reproduction in male and female rats concurrent with systemic toxicity. Significant effects on reproductive endpoints were repeatedly observed across multiple study designs. These findings include reduced mean live litter size, altered estrous cyclicity, reduced ovary weights and ovary histology. Increased uterine weight was observed in the uterotrophic assay, as was accelerated sexual maturation in immature animals in the female pubertal assay. Alterations in male reproductive parameters, including effects on multiple accessory organs, sperm production, and transport, were also identified in all studies where evaluated. Reductions in parental body weight are insufficient to be the primary cause for the observed changes in reproductive endpoints.

Reproductive endpoints observed in these studies appear to be reproducible and occurred at exposure concentrations that did not induce marked systemic toxicity or excessive changes to body weight parameters. Although there is no existing toxicokinetic information for TPP, findings in the uterotrophic assay and female pubertal assay provide evidence of the relevance to humans. In addition a SCL of 1.5% was determined from existing reproductive toxicity data with TPP and validated against data from existing reproduction studies conducted with TPP-derived substances that contain TPP as an impurity.

2.3 Current harmonised classification and labelling

TPP is not currently listed in the Annex VI of CLP Regulation and was not listed in Annex I of Directive 67/548/EEC. TPP does not currently have a harmonised classification by all manufacturers, importers, and downstream users.

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

TPP is not currently listed in Annex VI, Table 3.1 of the CLP Regulation.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

TPP is not currently listed in Annex VI, Table 3.2 of the CLP Regulation.

2.4 Current self-classification and labelling

TPP (CASRN 121158-58-5; EC 310-154-3) is listed on the CLP inventory with the following classifications: Skin Irrit. 2 (H315) or Skin Corr. 1A (H314); Eye Irrit. 2 (H319) or Eye Dam.1 (H318); Repr.1B (H360) or Repr. 2 (H361); Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410).

TPP (CASRN 121158-58-5; EC 310-154-3) was registered under REACH. The classification in one REACH registration dossier is: Skin Irrit. 2 (H315); Eye Irrit. 2 (H319); Repr.1B (H360); Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410). The classification in the other REACH registration dossier is: Skin Irrit. 2 (H315); Eye Irrit. 2 (H319); Repr. 2 (H361); Aquatic Chronic (H410).

The current self-classification of TPP is: Skin Irrit. 2 (H315); Eye Irrit. 2 (H319); Repr.1B (H360); Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410). A Specific Concentration Limit of 1.5% is proposed for the reproductive toxicity endpoint.

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Signal word: Danger

Hazard pictogram: GHS08: health hazard

Hazard statements: H360f: May damage fertility.

Precautionary statements:
P201: Obtain special instructions before use.

P202: Do not handle until all safety precautions have been read and understood.

P308+P313: IF exposed or concerned: Get medical advice/attention.

P405: Store locked up.

P501: Dispose of contents/container to..

2.4.2 Current self-classification and labelling based on DSD criteria

Indication of danger:
T - toxic

R-phrases:
R60 - may impair fertility

S-phrases:
S36/37- wear suitable protective clothing and gloves

S45 - in case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

S53 - avoid exposure - obtain special instructions before use

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

This proposal for harmonized classification and labeling is being submitted for tetrapropenyl phenol (TPP, EC No. 310-154-3) as it fulfills the criteria for classification set out in Annex I to the CLP Regulation in the following hazard class (Article 36(1) of the CLP Regulation): Reproductive toxicity, Category 1B.

There is no entry in Part 3 of Annex VI for this substance and justification for action needed at the Community level is based on the substance classification in a hazard class of highest concern (i.e. carcinogenicity, germ cell mutagenicity, reproductive toxicity, or respiratory sensitizer).

RAC general comment
Substance identity: The substance is a complex mixture of branched alkyl-substituted phenols, the majority of which are expected to be substituted at the 4- (para) position on the phenol ring. However it is expected that there will also be smaller amounts of 2(ortho)- and 3(meta)- substitution. The alkyl substituent is primarily branched C12 (dodecyl) with an unspecified branching pattern. The harmonised classification will apply to any substance which predominantly contains C12 (branched) alkyl-substituted phenols. For the purposes of this opinion, the substance is called Phenol, dodecyl-, branched (TPP). It is proposed that the Annex VI entry will also specify, under international chemical identification, Phenol, 2-dodecyl-, branched; Phenol, 3-dodecyl-, branched; Phenol, 4-dodecyl-, branched together with the alternative identifier Phenol, (tetrapropenyl) derivatives. The Dossier submitter (DS) proposed harmonized classification only for reproductive toxicity. Therefore only that proposal is considered in this opinion. However, RAC adopted an opinion on harmonised classification for additional hazard classes based on a separate CLH dossier for TPP submitted by another DS (SI Group-UK, Ltd). Therefore, the entries in the tables of this opinion for skin irritation, eye irritation and acute and chronic aquatic toxicity are those adopted by RAC, based on the other closely related opinion on TPP. References used in this opinion are given in full in the background Document (BD)

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Phenol, dodecyl-, branched

Tetrapropenylphenol (TPP)

The identifiers “Tetrapropenyl phenol” and “TPP” are commonly used to describe this UVCB substance. The term “TPP” is used throughout this report and should be considered equivalent to Phenol, dodecyl, branched. These terms have been recognized as equivalent in the OECD ICCA SIAR/SIAP (SIAM 22, Paris, April 18-21, 2006) dossier for this UVCB substance, and the chemical identities have been agreed among the SIEF for EC 310-154-3.

Table 5: Substance identity

EC number:	310-154-3
EC name:	Phenol, dodecyl-, branched
CAS number (EC inventory):	121158-58-5
CAS number:	121158-58-5
CAS name:	Phenol, dodecyl-, branched
IUPAC name:	Phenol, alkyl branched (species comprising decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl,substituents)
CLP Annex VI Index number:	Not listed in Annex VI
Molecular formula:	$C_{15}H_{24}O$ to $C_{21}H_{36}O$ (UVCB)
Molecular weight range:	MW range is 220 – 304. MW of C12 alkyl derivate homolog is 262.43

Alternative identifiers for this same substance are commonly used. The most common identifier is CASRN 74499-35-7; Phenol, (tetrapropenyl) derivatives. This CASRN is not listed on the EINECS inventory, and most manufacturers have used the descriptor CASRN 121158-58-5 to identify their substances. A more descriptive identifier could be: phenol, alkylation products with C10-C15 branched olefins derived from oligomerisation.

Alternative identifiers may include:

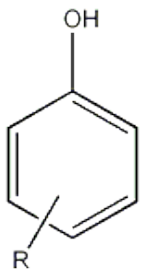
CASRN 74499-35-7; Phenol, (tetrapropenyl) derivatives

CASRN: 27193-86-6; Phenol, dodecyl

CASRN 104-43-8; Phenol, 4-dodecyl

Structural formula:

As the substance is a UVCB, this structural formula is for illustration only:



(R = C9/C10/11/12/13/14/15 branched alkyl nominally referred to as "tetrapropenyl-derivatives")

1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Phenol, alkyl branched (species comprising nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl constituents) CASRN 121158-58-5 EC 310-154-3	100%	Not applicable since UVCB	UVCB constituents identified by GC-MS and LC-MS
Phenol, C5-8, branched	4.6%	2 – 6%	
Phenol, nonyl-, branched	1.3%	0.5 – 2%	
Phenol, decyl-, branched	3.1%	2 – 7%	
Phenol, undecyl-, branched	14.2%	10 – 20%	
Phenol, dodecyl-, branched	44.0%	40 – 60%	This is the same substance assigned EC 121158-58-5, so it is not assigned as a single constituent
Phenol, tridecyl-, branched	14.0%	10 – 20%	
Phenol, tetradecyl-, branched	7.8%	5 – 15%	
Phenol, pentadecyl-, branched	5.1%	3 – 8%	
Phenol, C16+, branched	3.0%	1 – 5%	
Phenol	0.5%	0.1 – 0.5%	Not relevant for hazard classification; included for completeness
Alkenes, C10-14-branched and linear, C12-rich	2.5%	2 – 5%	Not relevant for hazard classification; included for completeness

Current Annex VI entry: Not applicable

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Not applicable	-	-	None

Current Annex VI entry: Not applicable

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None	-	-	-	None

Current Annex VI entry: Not applicable

1.2.1 Composition of test material

TPP has been tested over a period of time using material from a number of sources. The test data are considered to be based on samples of the substance representative of the description provided above as a UVCB. The key variations between sources will relate to the molecular weight and degree of branching of the alkyl group. The positioning of the alkyl chain on the phenol will be predominantly "para," but other positions are possible.

Supporting mechanistic studies were conducted with TPP, "purified" TPP and the calcium salt of TPP. Additional information is provided in Section 4.11.3.

In addition to the test data for TPP, additional test data are provided to further validate the Specific Concentration Limit (SCL). The SCL was validated with test materials representative of UVCB substances manufactured from TPP. These test substances are "TPP-derived" substances, and they contain TPP as an impurity. TPP present in these substances as manufactured ranges from 2.5 to 26 wt% TPP. The oligomers present in these substances are moderate to high molecular weight UVCBs (> 500 MW) and are expected to have limited bioavailability because of their molecular configurations, lack of available reactive sites and comparatively high molecular weights.

TPP-Derived Substances (Supporting SCL Validation)

EC No.	EC Name	Percent TPP	Remarks
455-880-2	reaction mass of: calcium bis(C10-14 branched alkylsalicylate); calcium bis(C18-30 alkyl salicylate); calcium bis(C10-14 branched alkyl phenolate); calcium bis(C18-30 alkyl phenolate); lubrificating oil (C15-30)	2.5%	Substance listed on ELINCS This complex UVCB substance has three basic manufacturing steps: 1. Carboxylation with CO2, 2. Neutralization of the resulting carboxylic acid intermediate with lime, and 3. Vacuum distillation to remove the majority of the unreacted TPP in its free phenol form. Most of the higher molecular weight alkylphenol remains behind. The Carboxylation reaction is performed using equal parts of TPP (310-154-3) plus a substantially linear higher molecular weight alkylphenol (272-232-2). During the course of this reaction, significant residual alkylphenols remain in the form of free phenols and their corresponding calcium salts.

EC No.	EC Name	Percent TPP	Remarks
415-930-6	reaction mass of: Ca salicylates (branched C10-14 and C18-30 alkylated); Ca phenates (branched C10-14 and C18-30 alkylated); Ca sulfurised phenates (branched C10-14 and C18-30 alkylated)	3.8%	Substance listed on ELINCS This complex UVCB reaction mass was prepared in three basic manufacturing steps: 1. Carboxylation with CO2, 2. Neutralization of the resulting carboxylic acid intermediate with lime, and 3. Reaction with elemental sulfur to crosslink the various intermediate "phenato" species formed during the Carboxylation/neutralization steps. The Carboxylation reaction is performed using equal parts of TPP (310-154-3) plus a substantially linear higher molecular weight alkylphenol (272-232-2). During the course of the sulfurization reaction, residual alkylphenols are chemically consumed resulting in a significant reduction of all free phenols and their corresponding calcium salts. These unreacted alkylphenol species are present in the final product. Because they are incidental, they were not specifically described in the ELNCS name given above during the course of its EU notification under the NONS program.
272-234-3	Phenol, dodecyl-, sulfurized, carbonates, calcium salts, overbased	6.7%	Substance listed on EINECS This complex UVCB substance was registered under REACH. This substance is manufactured in two fundamental reaction steps: 1. Sulfurization of the alkylphenol known as TPP in the presence of lime, and 2. Overbasing the sulfurized oligomers in the presence of CO2 and excess lime. The sulfurization reaction is not 100% efficient resulting in the presence of modest amounts of unreacted TPP as a mixture of the free phenol and its corresponding calcium salt. These incidental TPP residues remain in the desire product.

EC No.	EC Name	Percent TPP	Remarks
430-180-1	reaction mass of: calcium bis(C10-14 branched alkyl salicylate); calcium bis(C18-30-alkyl salicylate); calcium C10-14 branched alkylsalicylato-C18-30-alkyl salicylate; calcium bis (C10-14 branched alkyl phenolate); calcium bis (C18-30-alkyl phenolate); calcium C10-14 branched alkylphenolato-C18-30-alkyl phenolate; C10-14 branched alkyl phenol; C18-30-alkyl phenol	26%	This UVCB substance is manufactured in two basic steps: 1. Carboxylation with CO2, and 2. Neutralization of the resulting carboxylic acid intermediate with lime. The Carboxylation reaction is performed using equal parts of TPP (310-154-3) plus a substantially linear higher molecular weight alkylphenol (272-232-2). During the course of this reaction, significant residual alkylphenols remain in the form of free phenols and their corresponding calcium salts. These unreacted alkylphenol species are present in the desired product as described in the ELNCS name given during the course of its EU notification under the NONS program.

1.3 Physico-chemical properties

The data are pooled from manufacturers and are considered representative of the substance.

Note that certain key properties such as water solubility and partition coefficient will vary slightly according to the degree of branching and molecular weight. The partition coefficient has been measured at different times and on different batches and has been determined to be in the range of Log Kow 7.14 – 7.96.

The physico-chemical properties do not impact classification directly, and there are no hazardous properties based on physical effects. The substance is not dangerously reactive, explosive or flammable.

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Liquid	Oronite Additives (1993); Morris (1997);Tremain andAtwal (2010) Test material: TPP	Appearance visually assessed
Melting/freezing point	-9 °C (± 3 °C).	Chevron (2005) Test material: TPP	Measured of the pour point method (more appropriate than melting point) using ASTM D5950
Boiling point	189-270 °C	Chevron (2005) Test material: TPP	Thermogravimetric analysis method of boiling point range.
Relative density	0.9415 at 20 °C	Oronite Additives (1993) Test material: TPP	Measured using ASTM D1298
Vapour pressure	0.011 Pa at 25 °C	Tremain and Atwal (2010) Test material: TPP	Measured using method compatible with Method 104 specified in the OECD Guidelines for Testing of Chemicals (2006).
Surface tension	42.2 mN/m (90% saturated solution) at 22.0 ± 0.5°C	Woolley and O'Connor (2010) Test material: TPP	Measured using the method specified in the OECD Guideline 115 and EU Method A.5.
Water solubility	1.54 mg/l of solution at 20.0 ± 0.5°C	Mullee (2004) Test material: TPP	Measured using the flask method outlined in EU Method A.6 and OECD Guideline 105 (1995)
Partition coefficient n-octanol/water	log Kow 7.14 (slow-stir method)	Dutta (2003) Test material: TPP	Measured using a slow stir method (25°C) Modeled using KOWWIN v1.67
Flash point	150 °C	Woolley and O'Connor (2010) Oronite Additives (2003) Test material: TPP	Measured using a closed cup equilibrium method compatible with Method A.9 (2008).
Flammability	Not applicable	Not applicable	Study not technically feasible
Explosive properties	Not expected	Not applicable	Estimated by structural analysis
Self-ignition temperature	384 ± 5°C	Woolley and O'Connor (2010) Test material: TPP	Measured using Method A.15 (May 2008)
Oxidising properties	Not expected	Not applicable	Estimated by structural analysis
Granulometry	Not applicable	Not applicable	Substance is a liquid
Stability in organic solvents and identity of relevant	Stable	Not applicable	In accordance with Column 2 adaptation statement of REACH

degradation products			Annex IX, information requirement section 7.15, this study does not need to be conducted since the stability in organic solvents is not considered to be critical.
Dissociation constant	pKa ~ 10	CompuDrug (2010) Test material: Phenol, (tetrapropenyl) derivative	The dissociation constant (pKa) of a representative structure of Phenol, (tetrapropenyl) derivative was estimated using the pKalc function of PALLAS estimation software program. The pKa was determined to be 9.87. In accordance with Column 2 adaptation statement of REACH Annex IX, information requirement section 7.16, this study does not need to be conducted since the stability in organic solvents is not considered to be critical.
Viscosity	450 cSt at 40°C 9 cSt at 100 °C	Oronite Additives (1993) Test material: TPP	Measured using methods ASTM D445 and ASTM D2161.

2 MANUFACTURE AND USES

2.1 Manufacture

Manufacturing details of the registered substance are described here.

IU Number	Identified Use Name (IU)	Sector of End Use (SU)	Process category (PROC)	Environmental release category (ERC)
1	Manufacture	3, 8	1, 8b, 15	1

Phenol, dodecyl-, branched is a UVCB substance used in chemical synthesis processes, which convert this alkylphenol into other chemical derivatives. As such, we provide a minimal working description of the manufacturing process for the purposes of this CLH proposal.

Phenol, dodecyl-, branched is manufactured in a dedicated continuous processing plant using a pressurized fixed bed flow-through reactor system into which olefin and phenol are injected at constant rates. The exothermic alkylation reaction is promoted using an acidic solid phase ion exchange resin catalyst affording the desired crude alkylphenol. The alkylation reaction is essentially complete by the time these reactants leave the reactor assembly, at which point the continuous crude flow is pumped to an adjacent continuous feed distillation column for final

purification to meet control specifications. At this process stage, unreacted phenol and unreacted olefin are removed overhead for later recycle. The desired bottoms are recovered and stored for later use in the manufacture of diverse chemical derivatives of this alkylphenol.

The substances manufactured from TPP contain residues of the substance (impurity), and it is important that the presence of TPP is identified in these products if present at concentrations above thresholds of concern.

2.2 Identified uses

The substance has the following registered use:

IU Number	Indentified Use Name (IU)	Sector of End Use (SU)	Process category (PROC)	Environmental release category (ERC)
1	Chemical industry; chemical used in synthesis; use of monomer for synthesis of polymer	3, 8	1, 2, 3, 4, 8b, 15	6c

The substance is synthesized and used under predominantly closed industrial chemical processes. The only registered use is in the chemical industry as an intermediate/monomer for the synthesis of higher molecular weight substances and polymers.

The substance is not supplied to the general public.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

No hazards are identified with respect to physico-chemical properties. Therefore, the CLH report does not propose a hazard classification based on physico-chemical properties.

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Not applicable	-	-	-

3.1 *Physico-chemical Properties*

3.1.1 Summary and discussion of physico-chemical properties

No hazard classification is proposed.

3.1.2 Comparison with criteria

No hazard classification is proposed.

3.1.3 Conclusions on classification and labelling

No hazard classification is proposed.

4 HUMAN HEALTH HAZARD ASSESSMENT

Only the properties relevant to the proposed reproductive toxicity classification are described in detail.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

No toxicokinetic studies are available that directly address absorption, metabolism, distribution and elimination (ADME) properties of TPP. However, information is available from existing toxicology studies to infer potential toxicokinetic properties.

Systemic availability of TPP depends on absorption across body surfaces. Factors that affect this process include water solubility, lipophilicity (partition coefficient), the dissociation constant (pKa), and molecular size. The physical state of the substance is an oily liquid under ambient conditions and is very lipophilic, with an estimated log Kow of 7.14. The estimated water solubility is 1.54 mg/L for the main component.

4.1.1 Non-human information

Oral Exposure

Based on its high lipophilicity and low water solubility, TPP is expected to be absorbed into and through the cell membrane, to be retained in the body, and to have a potential to be distributed in

the body. The liver is expected to be the primary organ to receive and metabolize the substance, making it more soluble by oxidation and conjugation and releasing the more polar compound into the bile for elimination via the gastrointestinal tract. Systemic effects in subchronic toxicity studies confirm that TPP is absorbed and distributed after ingestion.

Whole body distribution is supported in the key study for reproductive toxicity with oral exposure (Edwards *et al.*, 2012), which found effects in both adults and neonates. Further studies are required to separate reproductive effects from direct effects on the neonate and to better understand distribution of the substance in the pregnant dam and fetuses to confirm this.

Dermal Exposure

TPP was evaluated *in vitro* in both rat and human cadaver skin to determine the percutaneous penetration of radiolabeled TPP (OECD 438; Bernard, 2012a). Dermal absorption of radiolabeled TPP was also evaluated *in vivo* using a rat model (OECD 427; Bernard, 2012b). The *in vivo* animal, *in vitro* animal and *in vitro* human data can be combined and evaluated to obtain an estimate of the absorption of TPP in humans (*OECD Guidance Notes on Dermal Absorption. Draft 22 October 2012).*

Dermal absorption and subsequent bioavailability following human exposure to TPP is expected to be low. [^{14}C]-TPP is absorbed at ~3% of the applied dose. This rate was not significantly altered by concentration (0.01, 0.1, or 1.0%) or exposure duration (1-72 hr) utilizing the most conservative values by adding the absorbed dose and the chemical remaining in the application site and surrounding skin.

Inhalation Exposure

This substance only exists in liquid form. The substance vapor pressure (0.011 Pa at 25 °C) suggests that the substance is unlikely to be inhaled. Thus exposures via inhalation that lead to absorption through the respiratory system are unlikely. No studies have been performed.

Notes on Formulations

The absorption of the chemical may be influenced by the other components present when the substance is supplied. Market product concentrates are most likely to contain less than 1% of TPP in the oligomer/polymer products that are themselves further diluted in base oil. This substance is unlikely to be present in finished lubricants for market at levels > 0.1%.

4.1.2 Human information

No data are available.

4.1.3 Summary and discussion on toxicokinetics

The toxicokinetic properties of TPP have not been directly studied in vivo, however the ADME can be inferred based on the physical chemical properties of TPP and the available animal dataset.

With respect to molecular weight (234-304), water solubility (1.54 mg/L), and Log Kow (7.14), tetrapropenyl phenol is expected to be absorbed in the gastrointestinal tract after oral administration. Molecular weight values less than 500 are most suitable for oral absorption, however, ingestion is not expected to be a major route of exposure for this chemical due to use patterns.

Using OECD guidance on dermal absorption, *in vivo* animal, *in vitro* animal and *in vitro* human data were evaluated in combination to obtain an estimate of the likely dermal absorption of TPP in humans. Absorption via skin is expected to be low (~3%).

Inhalation exposure is of negligible relevance due to the low vapour pressure (<0.1 Pa at 20°C) and use pattern.

Once absorbed, extensive distribution can be assumed based on the target tissues identified in subacute toxicological studies. The compound appears to be eliminated from tissues, based on recovery from tissue effects seen in a subacute oral study (Harriman, 2004) and in an acute dermal study (Randall and Robinson, 1978), tissues from higher levels in the repeat dose oral study showed prolonged tissue effects which may be related to retention in the body consistent with lipophilic properties of the substance.

From WHO Food Additives Series 46: Phenol and Phenol Derivatives, the test material is assumed to be subject to conjugation with sulfate and glucoronic acid, as well as ring hydroxylation and side chain oxidation at high dose levels. Elimination is assumed to be relevant and primarily via the urine.

4.2 Acute toxicity

No hazards are identified with respect to acute toxicity. The CLH report does not propose a hazard classification based on acute toxicity.

Table 11: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Not applicable	-	-	-

4.2.1 Non-human information

No hazard classification is proposed.

4.2.1.1 Acute toxicity: oral

No hazard classification is proposed.

4.2.1.2 Acute toxicity: inhalation

No hazard classification is proposed.

4.2.1.3 Acute toxicity: dermal

No hazard classification is proposed

4.2.1.4 Acute toxicity: other routes

Not applicable.

4.2.2 Human information

Not data are available.

4.2.3 Summary and discussion of acute toxicity

No classification is proposed.

4.2.4 Comparison with criteria

No classification is proposed.

4.2.5 Conclusions on classification and labelling

No classification is proposed.

4.3 Specific target organ toxicity – single exposure (STOT SE)

No hazards are identified with respect to target organ toxicity. The CLH report does not propose a hazard classification based on target organ toxicity.

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

No classification is proposed.

4.3.2 Comparison with criteria

No classification is proposed.

4.3.3 Conclusions on classification and labelling

No classification is proposed.

4.4 Irritation

4.4.1 Skin irritation

There is consistent self-classification for skin irritation of TPP. Therefore the CLH report does not request a harmonized classification for skin irritation.

Table 12: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Not applicable			

4.4.1.1 Non-human information

This CLH report does not request a harmonized classification for skin irritation.

4.4.1.2 Human information

No data are available

4.4.1.3 Summary and discussion of skin irritation

There is consistent self-classification for skin irritation of TPP based on available data.

4.4.1.4 Comparison with criteria

There is consistent self-classification for skin irritation of TPP.

4.4.1.5 Conclusions on classification and labelling

There is consistent self-classification for skin irritation of TPP. The substance is classified as a skin irritant according to the CLP and DSD criteria: H315 – Causes skin irritation (CLP) and R36: irritating to skin (DSD).

4.4.2 Eye irritation

There is consistent self-classification for eye irritation of TPP. Therefore the CLH report does not request a harmonized classification for eye irritation.

Table 13: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Not applicable	-	-	-

4.4.2.1 Non-human information

This CLH report does not request a harmonized classification for eye irritation.

4.4.2.2 Human information

No data are available.

4.4.2.3 Summary and discussion of eye irritation

There is consistent self-classification for eye irritation of TPP based on available data.

4.4.2.4 Comparison with criteria

There is consistent self-classification for eye irritation of TPP.

4.4.2.5 Conclusions on classification and labelling

There is consistent self-classification for eye irritation for TPP. The substance is classified as a serious eye irritant according to the CLP and DSD criteria: H319 – Causes serious eye irritation (CLP) and R38: irritating to eyes (DSD).

4.4.3 Respiratory tract irritation

No hazards are identified with respect to respiratory tract irritation. The CLH report does not propose a hazard classification based on respiratory tract irritation.

4.4.3.1 Non-human information

No classification is proposed.

4.4.3.2 Human information

No data are available.

4.4.3.3 Summary and discussion of respiratory tract irritation

No classification is proposed.

4.4.3.4 Comparison with criteria

No classification is proposed.

4.4.3.5 Conclusions on classification and labelling

No classification is proposed.

4.5 Corrosivity

No hazards are identified with respect to corrosivity. The CLH report does not propose a hazard classification based on corrosivity.

Table 14: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
Not applicable	-	-	-

4.5.1 Non-human information

No classification is proposed.

4.5.2 Human information

No data are available.

4.5.3 Summary and discussion of corrosivity

No classification is proposed.

4.5.4 Comparison with criteria

No classification is proposed.

4.5.5 Conclusions on classification and labelling

No classification is proposed.

4.6 Sensitisation

No hazards are identified with respect to sensitisation. The CLH report does not propose a hazard classification based on sensitisation.

4.6.1 Skin sensitisation

No classification is proposed.

Table 15: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Not applicable	-	-	-

4.6.1.1 Non-human information

No classification is proposed.

4.6.1.2 Human information

No data are available.

4.6.1.3 Summary and discussion of skin sensitisation

No classification is proposed.

4.6.1.4 Comparison with criteria

No classification is proposed.

4.6.1.5 Conclusions on classification and labelling

No classification is proposed.

4.6.2 Respiratory sensitisation

Table 16: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference
Not applicable	-	-	-

4.6.2.1 Non-human information

No classification is proposed.

4.6.2.2 Human information

No data are available.

4.6.2.3 Summary and discussion of respiratory sensitisation

No classification is proposed.

4.6.2.4 Comparison with criteria

No classification is proposed.

4.6.2.5 Conclusions on classification and labelling

No classification is proposed.

4.7 Repeated dose toxicity

Table 17: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Rat (Sprague-Dawley) 90 day study Oral: feed 0, 50, 100, 150, 200 mg/kg/day (Nominal in diet) Exposure: 91 -92 consecutive days (Male & Female) OECD Guideline 408 (Repeated Dose 90-day Oral Toxicity Study in Rodents)	NOEL: < 50 mg/kg/day Oral (dietary) administration based on: test material (based on body weight, food consumption, hematology, organ weights, macroscopic and microscopic effects) NOAEL: 100 mg/kg/day based on: test material (based on body weight, food consumption, hematology, organ weights, macroscopic and microscopic effects)	1 (reliable without restriction) Key study Experimental result Test material: TPP	Haas *et al.* (2012)
Rat (Crl:CD (SD)IGS BR) male/female Subacute (oral: gavage) 0, 5, 20, 60, 180, 300 mg/kg/day Exposure: 28 days (7 days/week) OECD Guideline 407	NOAEL: 60 mg/kg bw/day (actual dose received) (male/female) Based on: test mat. Organ weight and/or microscopic findings in the reproductive organs persisted to the recovery necropsy	1 (reliable without restriction) Supporting study Experimental result Test material: TPP	Harriman (2004)
Rat (Sprague-Dawley) male/female Subacute (oral: feed) 0, 500, 2500 and 5000 ppm in the diet (nominal in diet) Exposure: 28 day (7 days/week)	NOEL: 500 ppm in diet/feed (male/female) Based on: test mat. (Estimated approximately 25 mg/kg bw/day)	1 (reliable without restriction) Supporting study Experimental result Test material: TPP	Reyna and Thake (1988)
Rat (FDRL Strain) male/female Subchronic (oral: feed) 0, 0.05, 0.2 and 0.4% in the diet (Approximately 25, 100 and 200 mg/kg/day) (nominal in diet)	NOEL: 25 mg/kg bw/day in diet (0.05%) (male/female) Based on: test mat.	2 (reliable with restrictions) Supporting study Experimental result Test material: TPP	Vogin (1970)

Exposure: 90 day (7 days/week)			
Dog (Beagle) male/female Subchronic (oral:feed) 0, 0.05, 0.2 and 0.4% in the diet (Approximately 25, 100 and 200 mg/kg/day) (nominal in diet) Exposure: 13 week (6 days/week)	NOEL: > 200 mg/kg bw/day in diet (0.4%) (male/female) Based on: test mat.	2 (reliable with restrictions) Supporting study Experimental result Test material: TPP	Vogin (1970)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Dietary 90-Day Repeat Dose Toxicity Study in the Rat (Haas *et al.*, 2012).

Study Design

TPP was administered in the diet of Sprague-Dawley Crl:CD rats at dosage levels of 0, 50, 100, 150 and 200 mg/kg/day for a minimum period of 90 days according to OECD 408 guidelines (1998). Group sizes were ten per sex per dose level. This study was conducted to provide dose-selection guidance for a 2-generation reproduction study (Edwards *et al.*, 2012); as a result, not all parameters required in the OECD 408 test guideline were included. Note that semen analysis and estrous cyclicity, evaluated in the subsequent dietary route reproduction study, were not evaluated.

Summary of No Observed (Adverse) Effect Levels

NO(A)EL	Results
NOEL	< 50 mg/kg/day
NOAEL	100 mg/kg/ day

Results

Clinical Observations

- All study animals survived to scheduled necropsy
- No significant clinical observations

Food Consumption and Body Weight

- Dose-dependent decrease in food consumption in both males and females treated with TPP. This decrease was apparent in all TPP treated males during the first week of the study. The effects on food consumption in females were at a lower magnitude and with a later onset than with males.

  - Dose-dependent decrease in body weights compared to control in both males and females

- Week 10: (reduction compared to controls)
  - Males: 12.2%, 16.4%, 27.7%, and 34.9%
  - Females: 7.3%, 9.1%, 10.8%, and 16.1%
- Week 13: (reduction compared to controls)
  - Males: 11.7%, 17.7%, 30.4%, and 35.6%
  - Females: 10.4%, 11.8%, 14.3%, and 19.4%

  Note: authors consider the reduced body weights and weight gains to be a result of the reduced feed consumption

- Final body weights for males and females were significantly reduced in all TPP-exposed groups at 50-600 mg/kg/day

Hematology and Serum Chemistry:

- Decreased red blood cell (RBC) counts and hemoglobin in males treated at the top dose level tested, 200 mg/kg/day TPP
- Decreased white blood cell and absolute lymphocyte counts in both males and females treated at 200 mg/kg/day TPP
- Decreased mean alanine aminotransferase in males treated at 150 and 200 mg/kg/day
- Decreased mean cholesterol in females treated at 100, 150, and 200 mg/kg/day TPP

Gross Necropsy and Organ Weight Changes

- Small coagulating glands, prostate, and seminal vesicles noted in males treated at 150 and 200 mg/kg/day
- Small epididymides and testes in males treated at 200 mg/kg/day
- Increased mean adrenal gland weights (absolute, relative to final body weight, and relative to brain weight) in males treated at 100, 150, and 200 mg/kg/day
- Decreased mean prostate weight and seminal vesicles weight at 100, 150, and 200 mg/kg/day (lower relative to body weight at 150 and 200 mg/kg/day): higher relative testes weights at 100 and 150 mg/kg/day (lower absolute weight at 200 mg/kg/day).
- Increased mean testes weight in males treated at 100 and 150 mg/kg/day.
- Increased thyroid weights in males treated at 100, 150, and 200 mg/kg/day
- Decreased ovaries/oviducts weights in females treated at 100, 150, and 200 mg/kg/day
- Decreased uterine weights in females treated at 150 and 200 mg/kg/day

Histological Changes

- Adrenal cortical hypertrophy in males treated at 200 mg/kg/day

- Coagulating gland atrophy and prostate atrophy in males treated at 200 mg/kg/day
- Decreased secretion in the seminal vesicles in males treated at 150 and 200 mg/kg/day
- Renal tubular mineralization in males from the 200 mg/kg/day group

  Note: Finding was observed in 9/10 males at 200 mg/kg/day, in 3/3 and 3/3 at 50 and 100 mg/kg/day, but only in 1/10 male control rats. The kidneys of male rats dosed at 150 mg/kg/day were not evaluated
- Periportal hepatocellular vacuolation in males and females treated at 150 and 200 mg/kg/day
- Decreased corpora lutea in the ovaries in females at treated 150 and 200 mg/kg/day

Discussion and Conclusions

Females

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

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

Although severe food restriction in rats, < 70% of ad lib consumption, or sufficient to reduce body weight to < 70% of control body weight, has been shown to alter estrous cyclicity and/or decrease ovary weight (Chapin *et al.*, 1993, Seki *et al.*, 1997), the maximum reduction in female rat body weight in the TPP 90-day study, 81% of control, was insufficient to cause reproductive changes. Reductions in female body weight to 80% and 85% of control (Chapin *et al.*, 1993, Seki *et al.*, 1997) were shown to have no effect on reproductive performance or other reproductive parameters in rats.

Table 18: Key Findings: Effects on Female Reproductive Parameters (*Haas et al., 2012)*

Parameter (Females)	Dose Level (mg/kg/day)				
	0	50	100	150	200
Mean Absolute Organ Weights and Microscopic Findings (incidence)					
Mean Terminal Body Weight (g)	279	250*	246**	239**	225**

Mean Ovaries/Oviducts (g)	0.1289	0.1252	0.1021**	0.0947**	0.0772**
Ovaries – decreased presence of corpora lutea (5 or less)	1/10	1/10	1/10	4/10	7/10
Mean Uterus Weight (g)	0.74	0.76	0.74	0.56	0.50
Estrous Cycle: Diestrus	4/10	-	-	-	3/10
Estrous Cycle: Estrus	2/10	-	-	-	7/10
Estrous Cycle: Proestrus	4/10	-	-	-	0/10

Males

Findings at necropsy included small coagulating glands, prostate and seminal vesicles in the 150 and 200 mg/kg/day dose groups and small epididymides and testes in the 200 mg/kg/day dose groups. Reductions in absolute testes weight (by 36%) and in relative testes weight along with other changes in the testes included atrophy and hypospermia in the 200 mg/kg/day dose group. Reduced prostate and seminal vesicle weights (relative and absolute) were noted in the 100, 150 and 200 mg/kg/day treatment groups while testes weights in the 100 and 150 mg/kg/day groups were increased compared to control. These results are interpreted with caution, as male accessory reproductive organ weights are sensitive to changes in body weight (Rehm *et al.,* 2008).

Microscopic findings included hypospermia in the testes in 2/20 animals at the 100 mg/kg/day dose level hypertrophy of coagulating gland and atrophy of the prostate at 200 mg/kg/day. Decreased seminal vesicle secretions were seen in the 150 and 200 mg/kg/day dose groups as well. Renal mineralization, more commonly observed in females, was seen in male kidneys at all doses investigated.

Table 19: Key Findings: Effects on Male Reproductive Parameters (*Haas et al., 2012)*

Parameter (Males)	Dose Level (mg/kg/day)				
	0	50	100	150	200
Mean Absolute Organ Weights and Microscopic Findings (incidence)					
Mean Terminal Body Weight (g)	497	439**	409**	346**	320**
Mean Adrenal Glands (g)	0.0576	0.0596	0.0696	0.0729*	0.0893**

Parameter (Males)	Dose Level (mg/kg/day)				
	0	50	100	150	200
Mean Epididymides (g)	1.40	1.27	1.21**	1.04**	0.78**
Mean Prostate (g)	1.15	0.97	0.79**	0.53**	0.25**
Mean Seminal Vesicles (g)	2.06	1.83	1.57**	0.97**	0.36**
Mean Testes (g)	3.37	3.27	3.42	3.19	2.47**
Seminal Vesicles - Decreased Secretion	0/10	0/10	0/10	2/10	9/10

28-Day Oral Gavage Study in Rats (Harriman, 2004)

Study Design

In the 28-day study, Crl:CD (SD) IGS BR rats received doses of 0 (corn oil vehicle), 5, 20, 60, 180 and 300 mg/kg/day of TPP by oral gavage (5 mL/kg dosage volume). The protocol was designed and the study was conducted to meet or exceed the OECD 407, 1995. The 0 and 300 mg/kg/day groups contained 10 animals/sex (5/sex/group terminated at 28 days, remaining 5/sex/group terminated after 14-day recovery period), and the 5 through 180 mg/kg/day groups contained 5/sex/group. The study was designed to provide guidance for dose selection for the subsequent one-generation oral (gavage) reproduction study.

Summary of No Observed Adverse Effect Levels

End Point	NOAEL (mg/kg bw/day)
Toxicity (males/females) (Organ weight and/or microscopic findings in reproductive organs – [ovaries, seminal vesicles, prostate, coagulating glands, epididymides and/or testes])	60

Results

Body Weight

- Significant decrease in cumulative body weight gain, male and female, at 180 and 300 mg/kg/day, accompanied by slightly lower food consumption. Body weight gain effects dissipated during the recovery period.

  - Males: 42-45% (body weights 87% and 90% of control); Females: 14-21% *Note: Statistical significance for males only ($p<0.05$ or $p<0.01$)*

Hematology

- Decreased hemoglobin in females at 180 and 300 mg/kg/day
  - Females: 9-12%
  - Males: no effects

Serum Chemistry

- Increased triglycerides at 180 and 300 mg/kg/day
  - Females: 209% of control, both dosages
  - Males: no effect
- Decreased cholesterol at 180 and 300 mg/kg/day
  - Females: 56% and 53% of control, respectively
  - Males: no effect

  Note: There were no test article-related changes in serum chemistry parameters.

Organ Weights

- Adrenal: increased absolute weight
  - Males - 180 and 300 mg/kg/day (169% and 191% of control, respectively)
  - Females: no statistically significant increases
- Heart: decreased absolute weight and ratio relative to brain
  - Males: 180 and 300 mg/kg/day (85% and 81% of control, respectively)
  - Females: no effect
- Testes: decreased absolute weight and ratios relative to brain or body weights
  - Males: 180 and 300 mg/kg/day (85% and 58% of control value, respectively)
- Male Accessory Reproductive Organs (Coagulating Gland, Epididymides, Prostate, Seminal Vesicles: dose-responsive decrease at 180 and 300 mg/kg/day
  - Most severe organ weight reduction: seminal vesicles reduced to 33% and 21.5% of control weights, respectively
- Ovaries: dose-responsive decreased weight at 180 and 300 mg/kg/day
  - 76% and 72% of control, respectively
- Liver: dose-responsive increase
  - Males: 300 mg/kg/day (138% of control)

  - Females: 300 mg/kg/day (137% of control)
  - Coincided with increased incidence of centrilobular hepatocellular hypertrophy and periportal hepatocellular vacuolization.

Histological Changes

- Testes: maturation depletion (300 mg/kg/day; 4/5 vs. 0/5 control) and/or interstitial cell atrophy (180 and 300 mg/kg/day; 5/5 and 4/5, respectively, vs. 0/5)
- Prostate and Seminal Vesicles: decreased secretion (180 and 300 mg/kg/day; 5/5 at each dose level vs. 0/5 control)
- Ovaries: decreased Corpora Lutea (180 and 300 mg/kg/day; 2/5 and 3/5 vs. 0/5 control)
- Liver: centrilobular hepatocellular hypertrophy and/or periportal hepatocellular vacuolization
  - Males: 180 and 300 mg/kg/day (3-5/5 vs. 0/5 control)
  - Females: 180 and 300 mg/kg/day (4-5/5 vs. 0/5 control)
- Thyroid: follicular cell hypertrophy
  - Males: all doses (1-3/5 vs. 0/5 control)

Discussion and Conclusions

Repeated Dose Toxicity

There was overt toxicity at the top two doses evidenced by decreased cumulative mean body weight gains that resulted in mean lower body weights (statistically significant in males only, 13% and 10% reductions at 180 and 300 mg/kg/day, respectively).

Changes observed only in females included decreased hematocrit and hemoglobin, decreased serum cholesterol, and increased serum triglycerides. These changes were observed at 180 and 300 mg/kg/day in a dose-responsive pattern. Mean hemoglobin values (g/dL) were statistically significantly less than control values by 9-12% in females treated with 180 and 300 mg/kg/day.

A number of treatment-related organ changes were also observed. In males at 180 and 300 mg/kg/day, mean increases in absolute weights of the adrenal gland of 69% and 91% were observed. At 20 and 60 mg/kg/day there were no increases in adrenal gland weight, but at doses of 20 mg/kg/day and above there was an increased incidence (0/5, 0/5, 2/5, 2/5, 5/5 and 5/5 at doses of 0, 5, 20, 60, 180 and 300 mg/kg/day, respectively) and severity (from minimal to mild) of adrenal gland hypertrophy noted microscopically. There was no statistically significant increase in adrenal gland weight in females at any dosage.

Liver weights increased with dose, becoming statistically significant in male and females exposed to 300 mg/kg/day compared to controls. The increase in liver weights coincided with an increased incidence of animals with centrilobular hepatocellular hypertrophy (males: 0/5, 0/5, 2/5, 2/5 and 5/5, females: 0/5, NE, 0/5, 4/5 and 5/5 at doses of 0, 20, 60, 180 and 300 mg/kg/day, respectively) and periportal hepatocellular vacuolization (males: 0/5, 0/5, 0/5, 0/5 and 3/5, females: 0/5, NE, 0/5, 0/5 and 1/5 at doses of 0, 20, 60, 180 and 300 mg/kg/day, respectively).

The incidence in the number of male rats with follicular cell hypertrophy in the thyroid increased with dose (0/5, 1/5, 1/5, 2/5, 3/5 and 3/5 at doses of 0, 5, 20, 60, 180 and 300 mg/kg/day,

respectively) but these changes were not observed in females. Follicular cell hypertrophy tends to be a transient finding in rats and has limited relevance to human hazard identification.

Females

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

Males

Mean testes weights were statistically significantly reduced by 42% in males at 300 mg/kg/day; this reduction was accompanied by an increased incidence in the number of animals with germ cell depletion and interstitial cell atrophy. Mean testes weights were reduced by 15% in males at 180 mg/kg/day, and although the reduction was not statistically significantly different from control, it was accompanied by an increased incidence in the number of animals with interstitial cell atrophy (0/5, 0/5,0/5, 5/5, and 4/5) and depletion of mature germ cells (0/5, 0/5, 0/5, 1/5, 4/5). There was also a low (1/5) incidence of animals with microscopic degeneration of the seminiferous tubules in the testes at all dose levels, though this effect showed no dose-response over the 5 to 300 mg/kg/day dose range.

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

Table 20: Key Findings: Effects on Male Reproductive Parameters (*Harriman, 2004)*

Parameter	**Dose Level (mg/kg/day)***			
	0	60	180	300
Organ weights as percentage of control (absolute unless stated) and microscopic findings (incidence)				
Mean Terminal Body Weight (g)	100	99	87*	90*
Cumulative Body Weight Change (wk 0-4)	100	95	55**	58**
Mean Testes Weight	100	99	85	58**
Mean Testes Weight, relative	100	98	98	65**
• interstitial cell atrophy	0/5	-	5/5	4/5
• depletion of mature germ cells	0/5	-	1/5	4/5
Mean Epididymides Weight	100	102	71**	42**
Mean Epididymides Weight, relative	100	101	82	47**
• hypospermia*	0/5	-	0/5	3/5
• luminal debris	0/5	-	0/5	4/5

Parameter	Dose Level (mg/kg/day)*			
	0	60	180	300
Organ weights as percentage of control (absolute unless stated) and microscopic findings (incidence)				
Mean Seminal Vesicle Weight	100	91	33**	21**
Mean Seminal Vesicle Weight, relative	100	90	38**	24**
• decreased secretion	0/5	0/5	5/5	5/5
Mean Prostate Weight	100	99	44**	21**
Mean Prostate Weight, relative	100	97	50**	24**
• decreased secretion	0/5	0/5	5/5	5/5
[1] 5 and 20 mg/kg bw/day groups omitted from summary table; no relevant findings *p<0.05; **p<0.0l; "-" = Not examined				

28-Day Dietary Study in Rats (Reyna and Thake, 1988)

Study Design

TPP was administered to male and female Sprague-Dawley rats via the diet at concentrations of 0, 500, 2500, and 5000 ppm. Group sizes were 10 per sex. All rats were sacrificed at the end of four weeks of exposure. The study was conducted in general conformance with the Environmental Protection Agency Good Laboratory Practice Standards.

Summary of No Observed (Adverse) Effect Levels

End Point	NO(A)EL (ppm)
Toxicity (male and female)	500 ppm* – NOEL

* Estimated approximately 25 mg/kg/day

Results

Body Weight and Food Consumption

- Males: dose-dependent decreases in body weight gain, including body weight loss at 2500 and 5000 ppm (35.5% of control and 15.5 g of weight loss vs. 115 g weight gain in the control males)
- Males: dose-dependent decrease in food consumption at 2500 and 5000 ppm (71.2% and 50.7% of control, respectively)
- Females: dose-dependent decrease in body weight gain at 2500 and 5000 ppm (48.3% and 28.4% of control, respectively)
- Females: dose-dependent decrease in food consumption at 2500 and 5000 ppm (81.4% and 73.1% of control, respectively

Hematology

- Males: reduced reticulocytes, 56% and 30% of control, respectively, at 2500 and 5000 ppm
- Females: changes either slight, sporadic or within historical control ranges, and were not considered related to treatment

Serum Chemistry

- Males: increased blood urea nitrogen (5000 ppm), increased chloride (2500, 5000 ppm), decreased SGOT (5000 ppm), decreased SGPT (5000 ppm)
- Females: increased blood urea nitrogen (5000 ppm), increased Y-GT (5000 ppm), and decreased SGPT (5000 ppm)

Organ Weights

Note: Laboratory only reported those organs, which were statistically different from control as both absolute weights and organ weight/body weight ratios.

- Males: increased adrenal (5000 ppm)
- Females: increased liver weight (5000 ppm)

Gross Necropsy

- Males: small or atrophic prostate, seminal vesicles, and testes; abnormally soft testes (5000 ppm)

Histological Changes

- Males: testes (tubular hypoplasia); seminal vesicles (no secretion); prostate (decreased secretion, hypoplasia), epididymus (hypoplasia, decreased/absent sperm); (5000 ppm - all changes)

Discussion and Conclusions

Repeated Dose Toxicity

Severe toxicity occurred at the 5000 ppm dietary concentration in both sexes, evidenced by body weight loss or severely reduced body weight gain, with dose-dependent reductions in food consumption. Males were more severely affected than females. Interpretation of changes to blood/serum or organs is confounded by the degree of generalized systemic toxicity. Moderate toxicity occurred in both sexes at 2500 ppm. The NOEL for both males and females was 500 ppm.

Females

No findings related to female reproductive toxicity were reported; however, the female reproductive system was not evaluated thoroughly, as ovaries were not weighed and may not have been examined microscopically.

Males

Findings in the male reproductive tract included hypoplasia and/or reduced secretions in the testes and accessory reproductive organs at 5000 ppm.

13-Week Dietary Study in Rats and Dogs (Vogin, 1970)

Study Design

A commercial sample of tetrapropenyl phenol was evaluated in a 13-week oral feeding study in rats and dogs (non-guideline). TPP doses equivalent to 0, 27, 106 and 217 mg/kg/day in males, and 0, 28, 104 and 228 mg/kg/day in females were employed in the diet for 90 days to FDRL rats and beagle dogs. Group sizes were three males and three females per dose. The highest dose tested was 4000 ppm in the diet, equivalent to approximately 180 mg/kg/day (assuming a body weight of 11 kg and a daily food consumption of 0.5 kg).

Summary of No Observed Effect Levels

End Point	NOEL (mg/kg bw/day)
Rat (male and female)	25 (0.05% in the diet)
Dog (male and female)	200 (0.4% in the diet)

Results (Rat)

Food Consumption and Body Weight Changes

- Significant reductions in food consumption by females in mid- and high-dose groups and by males in the high-dose group
- Reduced mean body weight gains for these groups compared to controls but the differences in body weight gains were not significant
- No significant differences in terminal body weights

Clinical Chemistry

- No treatment related effects were identified in the hematology, clinical chemistry and urinalysis data

Organ Weights

- At the high dose, reductions in relative liver weight were seen in males (15%) and females (14%)

Histology

- No microscopic changes in the liver correlated to changes in organ weight

Results (D*og*)

There were no unscheduled deaths during the study. No treatment-related effects were observed in the clinical signs, body weight, food consumption, hematology, clinical chemistry, or urinalysis data of treated animals. Organ weight, macroscopic observations at necropsy, and microscopic evaluation of selected tissues (including testes and ovaries) were unremarkable.

Discussion and Conclusions

Rat

In the 90-day dietary study, doses equivalent to 0, 27, 106 and 217 mg/kg/day in males, and 0, 28, 104 and 228 mg/kg/day in females were tested. No treatment-related changes were observed at the low dose. There were significant reductions in food consumption by females in mid-and high-dose groups and by males in the high-dose group resulting in reduced mean body weight gains for these groups compared to controls, but the differences in body weight gains were not significant. Similarly there were no significant differences in terminal body weights.

No treatment related effects were identified in the hematology, clinical chemistry and urinalysis data.

At the high dose, reductions in rat relative liver weight were seen in males (15%) and females (14%) but there were no microscopic changes in the liver. A reduction in mean ovaries weights in females by (21%) was observed in the 4000 ppm dose level. No microscopic findings were reported for the ovaries. The LOEL for effects on the female reproductive system was 0.40%.

Dog

The 90-day oral dietary study in dogs, although limited in its reliability because there is no evidence of systemic toxicity at the high dose, suggests that either dogs are not as sensitive to the effects of tetrapropenyl phenol when given equivalent doses (mg/kg/day) as rats, or it may be that dogs and other species may not show these effects at all.

4.7.1.2 Repeated dose toxicity: inhalation

No data are available.

4.7.1.3 Repeated dose toxicity: dermal

No data are available.

4.7.1.4 Repeated dose toxicity: other routes

No data are available.

4.7.1.5 Human information

No data are available.

4.7.1.6 Other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

The no-observed-effect level (NOEL) for oral (gavage) administration of TPP to male rats for 28 days was less than 5 mg/kg/day due to a microscopic finding of follicular cell hypertrophy in the thyroid glands in one male from this group at the primary necropsy (Harriman, 2004). For female

rats, the NOEL was 20 mg/kg/day due to clinical observations noted at 60 mg/kg/day. Organ weight effects and microscopic findings in the adrenal gland were noted in males of the 20 mg/kg/day group and above. Follicular cell hypertrophy tends to be a transient finding in rats and has limited relevance to human hazard identification.

The microscopic findings in the thyroid and adrenal glands of the 300 mg/kg/day group did not persist to the recovery necropsy. Organ weight effects and microscopic findings in the liver were noted at 60 mg/kg/day and above and organ weights and/or microscopic findings in the reproductive organs (ovaries, seminal vesicles, prostate, coagulating glands, epididymides and/or testes) were noted at doses of 180 mg/kg/day and above. Because the organ weight and/or microscopic findings in the reproductive organs persisted to the recovery necropsy, the no-observed-adverse-effect level (NOAEL) was considered to be 60 mg/kg/day for males and females.

In the 90 day dietary study (Haas *et al.*, 2012), high dosage group rats (200 mg/kg/day) had significant increases in the ratio of liver to body weights and the male high dosage group rats also had a significant reduction in the ratio of testes to body weight. Additionally, histopathological examinations revealed that the only significant finding was testicular hypospermia occurring in six of 20 high dosage group rats. This finding appears to be related to the test material.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

The key parameter chosen for the 90 day dietary repeated dose toxicity study gave a NOAEL of 100 mg/kg/day. Effects were seen at 150 and 200 mg/kg, however these concentrations are above the cut off values for effects seen, as identified in the criteria set out in Directive 67/548/EEC and also Regulation (EC) no 1272/2008. Classification for repeated dose toxicity is not required according to DSD criteria.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

No classification is proposed based on available data. Effects were seen at 150 and 200 mg/kg, however these concentrations are above the cut off values for effects seen, as identified in the criteria set out in Directive 67/548/EEC and also Regulation (EC) no 1272/2008. Classification for repeated dose toxicity is not required according to DSD criteria.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

No classification is proposed based on available data.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

No hazards are identified with respect to target organ toxicity. The CLH report does not propose a hazard classification based on target organ toxicity.

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

No classification is proposed.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

No classification is proposed.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

No classification is proposed. There were no changes observed in any of the test species that indicate target organ effects.

4.9 Germ cell mutagenicity (Mutagenicity)

No hazards are identified with respect to mutagenicity. The CLH report does not propose a hazard classification based on mutagenicity.

Table 21: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
Not applicable	-	-	-

4.9.1 Non-human information

4.9.1.1 In vitro data

No classification is proposed.

4.9.1.2 In vivo data

No classification is proposed.

4.9.2 Human information

No data are available.

4.9.3 Other relevant information

No other relevant data are available.

4.9.4 Summary and discussion of mutagenicity

No classification is proposed.

4.9.5 Comparison with criteria

No classification is proposed.

4.9.6 Conclusions on classification and labelling

No classification is proposed.

4.10 Carcinogenicity

Table 22: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
Not applicable	No data	Not applicable	Not applicable

4.10.1 Non-human information

No data are available.

4.10.1.1 Carcinogenicity: oral

No data are available.

4.10.1.2 Carcinogenicity: inhalation

No data are available.

4.10.1.3 Carcinogenicity: dermal

No data are available.

4.10.2 Human information

No data are available.

4.10.3 Other relevant information

No data are available.

4.10.4 Summary and discussion of carcinogenicity

No classification is proposed.

4.10.5 Comparison with criteria

No classification is proposed.

4.10.6 Conclusions on classification and labelling

No classification is proposed.

4.11 Toxicity for reproduction

Table 23: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Rat (Sprague-Dawley) male/female Two-generation study Oral: feed 0, 1.5, 15 & 75 mg/kg/day (nominal in diet) Exposure: F0 males and females were exposed for 129-134 consecutive days and F1 males and females were directly exposed for 210-227 consecutive days. (The control and test diets were offered ad libitum to the F0 and F1 males and females for a minimum of 70 consecutive days prior to mating. The F0 and F1 males continued to receive the test and control diets throughout mating and through the day of euthanasia. The F0 and F1 females continued to receive the control and test diets throughout mating, gestation, and lactation through the day of euthanasia.) OECD Guideline 416 (Two-Generation Reproduction Toxicity Study) EPA OPPTS 870.3800 (Reproduction and Fertility Effects)	NOAEL (Parental toxicity) (F0): 15 mg/kg (nominal) (male/female) based on: test mat. (overall effects body weight; food consumption organ weights; histopathology.) NOAEL (Parental toxicity) (F1): 1.5 mg/kg (nominal) (male/female) based on: test mat. (overall effects body weight; food consumption organ weights; histopathology.) NOAEL (Reproductive toxicity) (F0 and F1): 15 mg/kg bw/day (nominal) (male/female) based on: test mat. (Based on decreased implantation sites, increased estrous cycle lengths and a reduction in mean epididymal sperm concentration.) NOAEL (Neonatal toxicity) (F1 and F2): 15 mg/kg bw/day (nominal) (male/female) based on: test mat. (Based on reductions in postnatal survival, lower offspring body weights and body weight gains (resulting in a delay in the mean age of balanopreputial separation, lower spleen and thymus weights, and post-weaning mortality) and an accelerated onset of vaginal patency.)	1 (reliable without restriction) Key study Experimental result Test material: TPP	Edwards et al. (2012)
rat (Sprague-Dawley) male/female One-generation study Oral: gavage 0, 5, 25 & 125 mg/kg/day (actual	NOAEL: (Reproductive toxicity) < 5 mg/kg bw/day* (male/female) based on: test mat. (based on mean estrous cycle length, implantation sites, reduced fertility, spermatogenesis, male/female histology, male/female organ weights)	1 (reliable w/o restriction) Supporting study Experimental result	Knapp (2006)

ingested) Exposure: Once daily for 73 consecutive days prior to mating. Dosing for the F0 males continued throughout mating and through the day prior to euthanasia, for a total of 138 to 143 doses. The F0 females continued to be dosed throughout mating, gestation and lactation, through the day prior to euthanasia, for a total of 115 to 128 doses. (Once daily) OECD Guideline 415 (One-Generation Reproduction Toxicity Study)	* The findings at 5 mg/kg/day, reduced secretion in the prostate (1/5) and reduced weight of the seminal vesicles/coagulating gland, were not replicated in the 2-generation study (Edwards *et al.*, 2012). NOAEL: (Systemic toxicity) 5 mg/kg bw/day (male/female) based on: test mat. (overall effects on organ weights and microscopic changes) NOAEL: (Neonatal toxicity) 5 mg/kg bw/day (male/female) based on: test mat. (overall effects on body weight)	Test material : TPP	
Developmental Toxicity			
Rat (Sprague-Dawley) Oral: gavage 0, 20, 100 and 300 mg/kg/day Exposure: days 6 - 15 of gestation (Females only, Once/day, Treated from Gestation Day 6 through 15) OECD Guideline 414 (Prenatal Developmental Toxicity Study)	NOAEL (maternal toxicity): 100 mg/kg bw/day NOAEL (embryotoxicity): 100 mg/kg bw/day NOAEL (fetotoxicity): 100 mg/kg bw/day NOAEL (teratogenicity): 100 mg/kg bw/day	1 (reliable without restriction) Key study Experimental result Test material: TPP	Schroeder, R (1987)

4.11.1 Effects on fertility

Data relevant to assessment of reproductive function and development were evaluated and compared to the classification criteria for reproductive toxicity as described within the CLP Regulation. Data were derived from reproduction, developmental toxicity, and repeated exposure toxicity tests conducted with TPP. Other relevant, supporting information was available from mechanistic studies summarized in Section 4.11.3. Based upon all relevant information, the evidence supported the following classification under the CLP regulation:

Toxicity to reproduction – fertility: Repr. 1B (May damage fertility)

Toxicity to reproduction – development: Not classified

In female rats, TPP affected reproductive function concurrent with mild to moderate changes to body weight insufficient to account for changes to reproductive parameters. This is interpreted to

demonstrate clear evidence of reproductive toxicity. The profile of male reproductive changes is consistent with the profile of changes reported for reproductive effects due to food restriction in male rats. For this reason, classification for fertility was not based upon the test data for males.

Developmental toxicity data support a conclusion of no classification. Effects upon prenatal and pre-weaning development occurred only in the presence of maternal toxicity sufficient to account for the effects. Changes to attainment of sexual maturation occurred only in the presence of direct postweaning exposure to TPP, and not in pups exposed only indirectly through the dam during gestation and lactation.

4.11.1.1 Non-human information

Two-Generation Dietary Reproduction Study (Edwards *et al.*, 2012)

Study Design

TPP was administered in the diet of Sprague-Dawley Crl:CD rats for a minimum of at least 70 consecutive days at concentrations of 0, 1.5, 15, and 75 mg/kg/day in accordance with OECD 416 test guidelines (1998). Group sizes were 30/sex for both generations. Parental animals were designated F0 and F1 for the first and second generations, respectively. F0 males and females were exposed for 129-134 consecutive days, and F1 males and females were exposed for 210-227 consecutive days.

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

Males were dosed daily until euthanasia; semen quality was evaluated at necropsy. Female rats were dosed through mating, gestation, lactation, until euthanasia. Estrous cyclicity was evaluated prior to mating in both generations. Due to altered timing of sexual maturation, anogenital distance was measured in the F2 offspring on postnatal (PND) 1.

Summary of No Observed Adverse Effect Levels

End Point/Generation	**NOAEL (mg/kg bw/day)**
Parental (F0)	15
Parental (F1)	1.5
Reproductive Toxicity (F0 & F1)	15
Neonatal Toxicity (F1 & F2, F2a)	15

Results

Reproductive Performance and Effects on Fertility

The numbers of pups born and live litter sizes were statistically reduced at 75 mg/kg/day for the F2a litters (13.4 versus 10.1 and 13.4 versus 9.5, respectively); these values were also lower, but not statistically significant, in the F1 and F2 litters. In F0 females of the 75 mg/kg/day exposure group, there was a statistically significant reduction in the mean number of implantation sites (13.2 vs. 15.0, control); F1 dams were not evaluated for implantation sites due to multiple gestations.

Pup growth was significantly reduced after postnatal day 4 standardization of litter sizes. For F1 litters, this was statistically significant in the 75 mg/kg/day from PND 7 - 21, and statistically significant at 15 mg/kg/day on PND 7. For the F2 litters in the 75 mg/kg/day group, pup body weights were significantly reduced on PND 1 and PND 7-21; F2a pup weights in this group were statistically lower than their control counterparts on PND 14 and 21.

The timing of sexual maturation was significantly altered in both the male and female offspring of the first generation in the 75 mg/kg/day exposure group. In male offspring, balanopreputial separation occurred later (47.1 days vs. 45.1 in controls) and at a lower body weight (226.4 g vs. 246.2 g); both differences were statistically significant. In females, vaginal patency occurred at a younger age (27.4 days vs. 32.4 days) and at a lower body weight (60.8 g vs. 112 g); both differences in females were statistically significant. The timing of sexual maturation is influenced both by hormonal and growth factors. In females, sexual maturation was accelerated, despite reduced growth rate. In the opinion of the study director, male sexual maturation was delayed due to delayed overall growth. As a result of these alterations to the timing of sexual maturation in the F1 offspring, anogenital distance was measured in the F2 offspring on PND 1 and evaluated as a function of the cube root of pup body weights; there were no differences across groups.

Female Reproductive Toxicity

Alterations to female reproduction included lengthened estrous cycles at 75 mg/kg/day, as well as an increase in the number of female rats in persistent diestrus; these changes were observed in both generations of adult female rats. Also observed at the high dose in both generations was a reduction in ovary weight accompanied by microscopic observations of reduced corpora lutea. Although body weight was reduced at the high dose level also, the degree of reduction in body weight in the F0 and F1 females, 88% and 76% of control body weight, respectively, was insufficient to account for the microscopic findings or reduced ovary weights, 71% and 62% of concurrent controls. Vaginal patency occurred earlier in the F1 offspring dosed at 75 mg/kg/day (27.4 days versus 32.4 days in controls).

During lactation, female body weights were significantly different from their concurrent control values in the 75 mg/kg/day group. In both generations, the pattern observed was heavier body weight than in control dams during the latter portion of lactation. As body weight during lactation can be influenced by litter size, with larger litters consuming more milk and thus a higher metabolic demand on the dam, the slightly smaller litter size may have provided a lower metabolic demand on the dams, thus lowering the food consumption needed (decreased at 75 mg/kg/day in both generations) and increasing the nutrition available for body mass of the dam (food efficiency was increased in the F0 and F1 dams for all three lactation periods).

Table 24. Key Findings: Effects on Female Reproductive Parameters (*Edwards et al., 2012)*

Parameter	**Dose Level (mg/kg/day)**

F0 and F1 females **(F1 offspring and F2/2a offspring)**	0	1.5	15	75
Mean Absolute Organ Weights and Microscopic Findings (incidence)				
Mean Terminal Body Weight (g)	325 413	323 389	321 383	286** (↓12%) 315** (↓24%)
Mean Body Weight (g) - Initiation of Mating (F1)	293	290	284	256** (↓12.6%)
Mean Ovaries Weight (g)	0.1202 0.1051	0.1210 0.0993	0.1142 0.1027	0.0846** (↓30%) 0.0651** (↓38%)
Ovaries – decreased presence of corpora lutea (5 or less)	1/30 6/28	0/27 2/28	0/30 3/30	6/28* 16/26*
Estrous Cycle Length (days)	4.3 4.3	4.3 4.2	4.5 4.6	5.4** 6.5**
Vaginal Patency (F1 only) (days)	32.4	32/2	32.4	27.4** (↓15%)
Persistent Estrus (>3 consecutive days)	1/30 0/30	0/30 0/30	0/30 0/30	0/30 2/27
Persistent Diestrus (>4 consecutive days)	0/30 8/30	0/30 4/30	6/30 9/30	12/30 20/27
Number Implantation Sites (measured in F0 only)	15.0	14.8	14.7	13.2* (↓12%)
Number Born	14.0 13.4/13.4	14.1 13.0/13.1	14.0 13.2/13.3	12.5 12.6/10.1*
Live Litter Size	13.8	13.9	13.7	12.2

Parameter **F0 and F1 females** **(F1 offspring and F2/2a offspring)**	**Dose Level (mg/kg/day)**			
	0	1.5	15	75
Mean Absolute Organ Weights and Microscopic Findings (incidence)				
	13.3/13.4	12.9/12.7	13.0/13.1	12.1/9.5*

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

Male Reproductive Toxicity

Test substance-related organ weight changes at 75 mg/kg/day consisted of lower weights of the left and right epididymides and cauda epididymides, prostate, and seminal vesicles/coagulating glands in F0 and F1 males, and lower left and right testes weights in F1 males. Mean epididymal sperm concentration was also lower in the 75 mg/kg/day dose group. These changes occurred concurrently to reduced body weight; the reduction in terminal body weight, 18.5% in the F0 and 28.4% in the F1 relative to the concurrent control, was of a similar magnitude to the reductions observed in the male accessory sex organ weights relative to the control values, 10.5% to 25%. Consequently there were few statistically significant differences from control when accessory reproductive organ weights were evaluated based upon their weights relative to control values.

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

Statistically significant delayed attainment of balanopreputial separation was noted in F1 males in the 75 mg/kg/day treatment group compared to controls in the presence of statistically significant lower mean body weight. The study director attributed the delay in attainment of this developmental landmark to the test-substance related lower mean body weight. There was no association between delayed preputial separation and failure to sire a litter.

Table 25: Key Findings: Effects on Male Reproductive Parameters (Edwards *et al.*, 2012)

Parameter **F0 and F1 Males**	**Dose Level (mg/kg/day)**			
	0	1.5	15	75
Mean Organ Absolute Weights (unless stated) and Microscopic Findings (incidence)				
Mean Terminal Body Weight (g)	616 791	623 814	611 754	502** (↓18.5%) 566** (↓28.4%)
Mean Body Weight (g) - Initiation of Mating (F1)	543	545	536	449** (↓17.3%)
Mean Testes Weight (g) Left	1.79 1.87	1.69 1.94	1.75 1.94	1.62* (↓5%) 1.74
Mean Testes Weight (g) Right	1.78 1.93	1.74 1.96	1.70 1.88	1.66 1.72** (↓11%)
Mean Epididymides Weight (g) Left	0.75 0.67	0.72 0.73	0.76 0.75* (↑12%)	0.63** (↓16%) 0.65
Mean Epididymides Weight (g) Right	0.79 0.76	0.76 0.80	0.79 0.77	0.68** (↓13.9%) 0.68** (↓10.5%)
Epididymis Sperm Concentration (x106/g) Left	365.2 310.1	333.6 339.4	357.3 350.2	288.5* (↓26%) 320.5

Parameter **F0 and F1 Males**	**Dose Level (mg/kg/day)**			
	0	1.5	15	75
Mean Cauda Epididymides Weight (g) Left	0.3666 0.3028	0.3339 0.3362	0.3755 0.3391* (↑12%)	0.2747** (↓25%) 0.2740
Mean Cauda Epididymides Weight (g) Right	0.3671 0.3349	0.3529 0.3588	0.3686 0.3372	0.2838** (↓23%) 0.2879** (↓14%)
Mean Cauda Epididymis Weight Relative to Body Weight (g/100g) Left	0.060 0.039	0.054 0.042	0.062 0.046**	0.055 0.049** (↑25%)
Mean Cauda Epididymis Weight Relative to Body Weight (g/100g) Right	0.060 0.043	0.057 0.045	0.061 0.045	0.057 0.052** (↑21%)
Mean Cauda Epididymis Weight Relative to Brain Weight (g/100g) Left	16.892 13.751	15.530 15.663* (↑14%)	17.450 15.825** (↑15%)	12.818** (↓24%) 13.116
Mean Cauda Epididymis Weight Relative to Brain Weight (g/100g) Right	16.885 15.253	16.483 16.714	17.137 15.720	13.235** (↓22%) 13.815
Mean Prostate Weight (g)	1.13 1.06	1.09 1.07	1.09 1.06	0.88** (↓22%) 0.92* (↓13%)
Mean Prostate Weight Relative to Brain Weight (g/100g)	51.959 48.133	50.983 49.916	50.633 49.262	41.039** (↓21%) 44.003

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

One-Generation Gavage Reproduction Study (Knapp, 2006)

Study Design

Three groups of male and female Sprague-Dawley Crl:CD rats (30 males and 30 females per group) were administered the test article daily by oral gavage for 73 consecutive days prior to mating. The one-generation study was designed to meet or exceed testing requirements of the OECD Guideline 415 (1983). Both sexes of the parental generation were treated with concentrations of 0 (corn oil vehicle), 5, 25 or 125 mg/kg/day by oral gavage (5mL/kg dosage volume). Males were dosed daily until euthanasia. Female rats were dosed through mating, gestation, and lactation until euthanasia. Estrous cyclicity was evaluated prior to mating and estrous cycle stage at necropsy; semen quality was evaluated at necropsy. Due to marked effects upon reproduction, selected offspring were retained post-weaning without dosing for evaluation of sexual maturation landmarks, vaginal opening or preputial separation.

Summary of No Observed Adverse Effect Levels

*The solitary finding of reduced prostate secretion at 5 mg/kg/day, upon which the NOAEL was based, was not replicated in the 2-generation study (Edwards *et al., 2012).* The study conclusion stated that there were no effects upon females at 5 mg/kg/day.

End Point/Generation	**NOAEL (mg/kg bw/day)**
Reproductive Toxicity (F0)	< 5* (males) 5 (females)
Systemic Toxicity (F0)	5
Neonatal Toxicity (F1)	5

Results

Systemic Effects

In the parental generation there were varying reductions in food consumption, body weight gain and terminal body weights at 25 and 125 mg/kg/day exposure groups. In males dosed at 125 mg/kg/day, there were increases in the weight of the adrenal glands with hypertrophy of the adrenal cortex. Mineralization of the kidney was also seen in males at 25 and 125 mg/kg/day and at 125 mg/kg/day in females. Mean absolute liver weight was reduced in males at 125 mg/kg /day and in females at 25 and 125 mg/kg/day; male liver weight relative to body weight was increased at 125 mg/kg/day.

Reproductive Performance and Effects on Fertility

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

21. Pup body weight gain was not statistically evaluated for the 125 mg/kg/day dose group due to the small sample size. Pups with potential exposure during gestation and lactation that were maintained on the study after weaning, but without postweaning dosing, had unaffected sexual maturation in the 5 and 25 mg/kg/day groups (no statistical evaluation of pups from the 125 mg/kg/day group due to insufficient litters).The adverse effect on fertility in the adult rats was accompanied by adverse microscopic changes in both male and female reproductive organs, adverse effects upon female cyclicity, and a reduction in epididymal sperm concentration (effects described below). The reduction in fertility and effects of reproductive organs occurred at doses that also induced other toxic effects, including reduced body weight gain and food consumption and changes in the adrenals, kidneys and liver. However, this toxicity to non-reproductive organs was insufficient to deem the reproductive findings as secondary non-specific effects.

Female Reproductive Toxicity

Mean absolute ovarian weight was significantly reduced in females dosed at 25 and 125 mg/kg/day (87% and 70%, respectively, of control). Microscopic evaluation of ovaries found an increase in ovarian cysts (15/30 vs. 4/30 in controls) and decreased corpora lutea (18/30 vs. 4/30 in controls) at 125 mg/kg/day. Uterine weight was unaffected, although this measure may not have been valid due to differences between exposure groups in proportions of rats that had produced litters. Microscopically, an increase in endometrial gland cysts (8/30 vs. 1/30 in controls) was detected at 125 mg/kg/day. For females of the 125 mg/kg/day group, a disproportionate number of females, many of which had mated but did not show evidence of pregnancy (implantation sites at necropsy), were in estrus at termination (16/30 vs. 3/30 controls). This finding mirrored changes to estrous cyclicity detected during weeks 7-10 of exposure: at the mid and high dose, estrous cycle length increased (4.9 and 5.2 days, respectively, vs. 4.4 days control). In the high dose group, 6/30 females and 16/30 females displayed persistent estrus or diestrus, respectively, and 6/30 females were essentially acyclic (vs. 0/30, 2/30, and 0/30 for each endpoint, respectively, for the control group).

Other findings included red material in the facial area, reductions in body weight (at 125 mg/kg/day, females were 90% of control body weight upon initiation of mating), reduced food consumption that mirrored the body weight gain reductions, and reduced food efficiency during the first weeks of exposure. Non-reproductive organ effects included decreased liver weight (increased relative to body weight) at 25 and 125 mg/kg/day, without microscopic changes and reduced kidney weight (increased relative to body weight) at 125 mg/kg/day with evidence of renal mineralization (7/30 vs. 1/30 control). Body weight decrements due to reduced food consumption have been demonstrated to affect female reproductive endpoints at severity levels (<70% body weight)(Chapin, 1993) beyond those observed in this study.

Although body weight was also reduced, the effect upon body weight (maximum decrease to 82% of control body weight at termination) was insufficient to cause the reduction in ovary weight. Studies in rats utilizing feed restriction have demonstrated that female body weight must be reduced to approximately 70% of control before ovary weight will decrease (Chapin, 1993; Seki *et al.*, 1997).

Table 21: Key Findings: Effects on Female Reproductive Parameters (*Knapp, 2006)*

Parameter	**Dose Level (mg/kg/day)**			
	0	5	25	125

Mean Absolute Organ Weights and Microscopic Findings (incidence)				
Mean Terminal Body Weight (g)	352	341	342	287** (↓18.5%)
Mean Body Weight (g) - Initiation of Mating	287.1	281.4	284.2	259.3** (↓0.9.3%)
Mean Ovaries Weight (mg)	143.8	141.7	125.6* (↓12.7%)	100.4** (↓30.2%)
Ovaries – cysts present	4/30	8/30	7/29	15/30*
Ovaries – decreased presence of corpora lutea (5 or less)	4/30	3/30	4/29	18/30*
Mean Uterus Weight (g)	0.56	0.59	0.56	0.65
Uterus – endometrial gland cyst present	1/30	-	0/29	8/30*
Mean Estrous Cycle Length (days)	4.4	4.6	4.9	5.2
Persistent Estrus (>3 consecutive days)[a]	0/30	0/30	0/30	6/30
Persistent Diestrus (>4 consecutive days)[a]	2/30	2/30	4/30	16/30
In estrus at Necropsy	3/30	-	3/29	16/30*
Fertility Index (Number Pregnant/number Mated)	28/30	24/30	25/30	4/30**
Mean Number Born	13.3	14.0	12.4	2.3**
Mean Live Litter Size	13.0	13.9	12.0	1.7**

Male Reproductive Toxicity

At doses of 5 mg/kg/day and greater, there was a significant decrease in mean seminal vesicle/coagulating gland absolute weight compared to control. Upon evaluation of these data, it was noted that the control weight was above the range for historical controls while the decrease in the seminal vesicle/coagulating gland weight at 5 and 25 mg/kg/day was reported to be within the range of historical control weights. Further analysis of organ weights revealed significant decreases in the seminal vesicle/coagulating gland weight relative to brain weight at all dose levels, and relative to body weight for the 125 mg/kg/day group.

Male accessory reproductive organ weights, particularly the seminal vesicles and prostate, are sensitive to body weight restrictions. Sensitivity may be due to the proportion of glandular luminal content (fluid) relative to organ mass (Chapin *et al.,* 1993; Rehm *et al.,* 2008). Consequently, effects upon male accessory organs are interpreted with caution. Given the absence of findings attributed to exposure in the two-generation study (Edwards *et al.,* 2012), the statistically significant differences at 5 mg/kg/day are not considered toxicologically relevant.

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

At the highest dose of 125 mg/kg/day, the mean testes and epididymides absolute weights were significantly decreased compared to controls. More informatively, significant decreases in testes and epididymides weights relative to brain weight were also observed at this dose level. Additionally, mean epididymal sperm concentration was significantly reduced from 365.2 x 10^6/g in controls to 303.2 x 10^6/g in the highest dose group. Also, there was a significant increase in the number of animals with microscopic findings of decreased secretions in the seminal vesicle glands compared to controls. As noted above, this may, in part, be associated with body weight effects.

Table 27: Key Findings: Effects on Male Reproductive Organs (*Knapp, 2006)*

Parameter	**Dose Level (mg/kg/day)**			
	0	5	25	125
Mean Organ Absolute Weights (unless stated) and Microscopic Findings (incidence)				
Mean Terminal Body Weight (g)	646	652	578** (↓10.5%	476 ** (↓26.3%)
Mean Body Weight (g) - Initiation of Mating	530	531.1	505.9	421.2** (↓20.5%)
Mean Testes Weight (g)	3.68	3.63	3.63	3.49 * (↓5.2%)
Mean Testes Weight Relative to Brain Weight (g/100g) Left/Right	85.85/ 85.46	84.35/84.55	84.14/83.99	83.06/79.70*
Mean Epididymides Weight (g)	1.49	1.49	1.43	1.23** (↓17.5%)
Mean Epididymides Weight Relative to Brain Weight (g/100g) Left/Right	34.77/ 34.58	34.51/ 35.05	33.21/ 32.77	29.13**/ 28.44**

Parameter	Dose Level (mg/kg/day)			
	0	5	25	125
Mean Cauda Epididymides Weight (g)	0.690	0.679	0.622**	0.516** (↓25.2%)
Mean Cauda Epididymides Weight Relative to Brain Weight (g/100g) Left/Right	16.26/15.9	15.82/15.74	14.37**/ 14.40*	11.84**/ 12.20**
Mean Epididymides (left) Sperm Concentration (x 10^6/gram)	365.2	342.5	316.7	303.2**
Mean SV/CG/Fluid Weight (g)	2.48	2.20** (↓11.3%)	2.10** (↓15.3%)	1.39** (↓44%)
Mean SV/CG/Fluid Weight Relative to Brain weight (g/100g)	115.59	102.28** (↓11.3%)	96.84** (↓16.2%)	64.52** (↓44.2%)
Seminal Vesicles – decreased secretion present	0/30	-	0/28	6/30 *
Coagulating Gland – decreased secretion present	9/30	12/29	20/28*	26/30*
Mean Prostate Weight (g)	1.04	1.01	1.03	0.67** (↓38.5%)
Mean Prostate Weight Relative to Brain Weight (g/100g)	48.43	47.24	47.90	31.22**
Prostate – decreased secretion present	6/30	13/29	20/28*	18/30*

4.11.1.2 Human information

No data are available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

In the key developmental toxicity study (Schroeder, 1987), TPP dosage levels were 0 (corn oil vehicle control), 20, 100 and 300 mg/kg/day on days 6 – 15 of gestation. The study was conducted according to the OECD 414 test guideline and Good Laboratory Practices. Dams were sacrificed on day 20 of gestation with examination of uterine contents. Fetuses were evaluated for external, visceral, and skeletal alterations.

Summary of No Observed (Adverse) Effect Levels

End Point	NO(A)EL (mg/kg bw/day)
Maternal toxicty	100 mg/kg bw/day
Developmental toxicity (teratogenicity, embryotoxicity, fetotoxicity)	100 mg/kg bw/day

Results

Maternal toxicity findings included reduced body weight gain and food consumption. Weight gain remained low during the post-dosing period, gestation days 16-20. Soft stool was also observed during and after the dosing period. No adverse effects were observed in animals of the 20 or 100 mg/kg/day exposure groups. There were no necropsy observations attributed to treatment.

At 300 mg/kg/day, developmental effects included an increase in resorption that resulted in a reduction in litter size. Growth retardation was evidenced by reduced mean fetal body weight and ossification. During the fetal external examinations, three fetuses from one high-dose litter had cleft palates and two fetuses (one fetus from each of two different litters) had similar digit reduction defects (i.e., ectrodactyly); however, the incidence of high-dose fetuses with external malformations (1.9% or 4/214) did not differ statistically from the control data.

No increase in visceral malformations or variations was observed in the high-dose group from the visceral examination.

At skeletal examination, the incidence with malformations at 300 mg/kg/day was statistically higher than the control data. The skeletal malformation seen with greatest frequency at high-dose was wavy rib. Although identified as a malformation, this observation is often considered a variation with evidence of postnatal repair (Carney and Kimmel, 2007). Additional skeletal alterations were curved scapula and/or scapular spine and abnormally shaped long bones (humerus, ulna, radius, femur), and a statistically significant increase in skeletal variations (primarily reduced ossification).

No treatment-attributed effects occurred at the dose levels that did not produce marked maternal toxicity. Developmental effects at 300 mg/kg/day were associated with significant effects to maternal health.

4.11.2.2 Human information

No data are available.

4.11.3 Other relevant information

Mechanistic Studies Related to Reproductive Toxicity

Table 28: Overview of mechanistic screening assays related to reproductive function

Method	Results	Remarks	Reference
Rat (Sprague-Dawley) female Uterotrophic Study Oral: gavage 0, 75, 125, 250, 500 mg/kg/day Positive Control: 17α-Ethynylestradiol (Dose: 0.2 mg/kg/day) Exposure: Once daily during study days 0-2 (three doses) OECD Guideline 440 (Uterotrophic Bioassay in Rodents Study)	NOEL< 75 mg/kg/day (female) based on: test mat. (Body Weight Changes) NOAEL< 75 mg/kg/day (female) based on: test mat. (Uterine Weights)	1 (reliable without restriction) Supporting study Experimental result Test material: TPP	Edwards et al. (2010a)
Rat (Sprague-Dawley) female Uterotrophic Study Oral: gavage 0, 75, 125, 250, 500 mg/kg/day (actual ingested) Positive Control: 17α-Ethynylestradiol (Dose: 0.2 mg/kg/day)	NOAEL < 75 mg/kg/day (female) based on: test mat. (Body Weight Changes) NOAEL < 75 mg/kg/day (female) based on: test mat. (Uterine Weights)	1 (reliable without restriction) Supporting study Experimental result Test material: (Purified	Edwards *et al.*, (2010b)

Method	Results	Remarks	Reference
Exposure: Once daily during study days 0-2 OECD Guideline 440 (Uterotrophic Bioassay in Rodents Study)		TPP, not as manufactured : impurities more polar than TPP removed chromatograp ohically)	
Rat (Sprague-Dawley) female Female Pubertal Assay Oral: gavage 10, 50, 200 and 800 mg/kg/day Exposure: Once daily for 20 consecutive days, postnatal days 22-41 U.S. EPA Good Laboratory Practice Standards (40 CFR Part 792), the OECD Principles of Good Laboratory Practice, the Japanese MAFF Good Laboratory Practice Standards, the standard operating procedures of WIL Research Laboratories, LLC.	NOAEL: 10 mg/kg bw/day (Female) based on: test mat. (vaginal patency, ovary weight)	1 (reliable without restriction) Supporting study Experimental result Test material: TPP (purified, not as manufactured) concentrated C12 homolog, >85%)	Knapp (2009a)
Rat (Sprague-Dawley) female Female Pubertal Assay	NOAEL: < 10 mg/kg bw/day (female) based on: research sample; distilled test material, not as manufactured (ovary weight)	1 (reliable without restriction)	Knapp (2009b)

Method	Results	Remarks	Reference
Oral: gavage 10, 50, 200 and 800 mg/kg/day Exposure: Once daily for 20 consecutive days, postnatal days 22-41 U.S. EPA Good Laboratory Practice Standards (40 CFR Part 792), the OECD Principles of Good Laboratory Practice, the Japanese MAFF Good Laboratory Practice Standards, the standard operating procedures of WIL Research Laboratories, LLC.		Supporting study Experimental result Test material: Distilled TPP, not as manufactured; concentrated C15 homolog >85%	
Rat (Sprague-Dawley) female Female Pubertal Assay Oral: gavage 5, 20, and 60 mg/kg/day Exposure: Once daily for 20 consecutive days, postnatal days 22-41	NOAEL: 20 mg/kg bw/day (female) based on: test material (vaginal patency, decreased corporal lutea, uterine hypoplasia, follicular cell hypertrophy)	1 (reliable without restriction) Supporting study Experimental result Test material: Calcium salt of TPP	Knapp (2007a)
Rat (Sprague-Dawley) female	NOAEL: < 60 mg/kg bw/day	1 (reliable without	Knapp (2007b)

Method	Results	Remarks	Reference
Female Pubertal Assay Oral: gavage 0, 60, 250, 1000 mg/kg/day Exposure: Once daily for 20 consecutive days, postnatal days 22-41	(female) based on: test material (vaginal patency, ovary weight, uterine weight, organ weight)	restriction) Supporting study Experimental result Test material: Calcium salt of TPP	
In vitro – androgen receptor binding assay Rat (Sprague-Dawley) prostate cytosol Test and control concentrations = 0.1nM – 1mM Ligand: R1881 Positive Control: dexamethasone Endocrine Disruptor Screening Program Test Guidelines OPPTS 890:1150	Mean IC_{50} = 92µM (TPP inhibition of R1881 binding to the androgen receptor) % RBA (relative binding affinity) = 1.57 x 10^{-7}%	1 (reliable without restriction) Supporting study Experimental result Test material: TPP	Thomas *et al.*, (2012a)
In vitro – uterine estrogen binding assay Rat (Sprague-Dawley) uterine cytosol Test and control concentrations = 0.1nM – 0.1mM Ligand: ^{3}H-E2 Positive Control: 19-norethindrone Negative Control:	Mean inter-day IC_{50} = 1100nM (TPP inhibition of ^{3}H-E2 binding to the estrogen receptor) % RBA (relative binding affinity) = 0.11%	1 (reliable without restriction) Supporting study Experimental result Test material: TPP	Thomas *et al.*, (2012b)

Method	Results	Remarks	Reference
octyltriethoxylsilane Endocrine Disruptor Screening Program Test Guidelines OPPTS 890:1250			

Uterotrophic Assay (Edwards *et al.*, 2010a)

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 supplier) and approximately 60 days of age at the beginning of test substance administration.

Summary of No Observed (Adverse) Effect Levels

End Point	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.
  - Blotted: 183% to 275% of control value
  - 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".

Uterotrophic Assay (Edwards *et al.*, 2010b)

Study Design

The test substance, purified TPP, 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 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 45 days of age at the time of ovariectomies (performed by the supplier) and approximately 60-64 days of age at the beginning of test substance administration.

Summary of No Observed Adverse Effect Levels

End Point	NOAEL (mg/kg bw/day)
Weight Changes (Female)	< 75
Uterine Weights (Female)	< 75

Results

Body Weight Changes

- Mean body weight in the 500 mg/kg/day group was significantly ($p<0.01$) lower (12.9%) compared to the vehicle control group on study day 3.
- Significant ($p<0.05$), test article-related mean body weight losses were noted in the 75 and 250 mg/kg/day groups during study days 0-3 compared to a mean body weight gain of 11 g in the vehicle control group.
- No body weight gain (0 g; not statistically significant) was noted in the 125 mg/kg/day group during study days 0-3.
- Differences from the vehicle control group did not occur in a dose-related manner and were not of a sufficient magnitude to result in remarkable changes in mean body weights.

- In the positive control group, mean body weight losses were noted throughout the treatment period, resulting in a 9.9% lower mean body weight compared to the control group on study day 3.

Macroscopic Examinations

- One animal in the 500 mg/kg/day group was found dead on study day 3. No remarkable internal findings were noted for this female.
- All other animals survived to the scheduled euthanasia on study day 3; no test substance-related internal findings in the uterus were observed at dosage levels of 75, 125, 250, and 500 mg/kg/day.

Uterine Weights

- Dose-related increase in absolute and relative uterine wet and blotted at all doses
    - Blotted: 184% to 251% of control value
    - Wet: 177% to 508% 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 a minimal reduction in body weight gain and 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) elicited the expected increases in uterine weights (wet and blotted). The interpretation of the laboratory was that TPP "demonstrated or mimicked biological activities consistent with agonism of natural estrogens".

Female Pubertal Assay (Knapp, 2009a)

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 ($p < 0.01$)**
    - **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).**

      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 ($p < 0.01$)
    - 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 ($\geq$ 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 estrus [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)
- 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)

- 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 uterus weights (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.

Female Pubertal Assay (Knapp, 2009b)

Study Design

TPP (distilled, laboratory-enriched, C12 homologs, not as commercially manufactured), 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

- Eleven of fifteen females (11/15, 73%) females in the 800 mg/kg/day group died following two to six 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 groups ($p<0.01$)
  - Mean day of attainment of vaginal opening was 32.5, 32.3, 29.3 and 29.2 days in the control, 10, 50, and 200 mg/kg/day groups, respectively (historical control range of 31,8 – 36.5 days).
    Note: acceleration was not observed in the F1 offspring in the one-generation reproduction gavage study at 25 mg/kg/day (Knapp, 2006); exposure post-weaning, which was intentionally not conducted in the 1-generation study, may be necessary to alter the timing of puberty.
- Decreased body weight at vaginal opening at 50 and 200 mg/kg/day groups ($p<0.01$)
  - Body weight (g) was 87.3 and 86.1 g at attainment across the dose groups versus 111.9 g in the control group

Estrous Cycle

- Mean first occurrences of estrus were earlier (31.6 and 32.1 days, respectively) than in the control group (34.4 days of age).
  Note: Estrus cycle lengths could only be determined for 5/15, 9/15, 12/15, and 10/15 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 the number of cycling animals available for evaluation. Estrous cycle lengths in females of this age are highly variable. Abnormal estrous cycles (≥5 consecutive days

of diestrus [D] or ≥3 consecutive days estrus [E]) were noted in 6/15, 6/15, 8/15 and 14/15 females in the control, 10, 5 and, 200 mg/kg/day groups, respectively.

Hormone Effects

- No test article-related effects on serum hormones were noted in the 10, 50 and 200 mg/kg/day groups; differences from the control group were not statistically significant.

Organ Weights

- Statistically significant reduction ($p<0.05$, $p<0.01$) in mean absolute ovary weights in the 10, 50 and 200 mg/kg/day groups.
  - Ovary weight (g) with oviducts was 0.0936, 0.0796, 0.0793, 0.603 g across the dose groups
- Statistically significant reduction ($p<0.05$, $p<0.01$) in mean absolute and relative uterus (wet and blotted) weights in the 10 and 200 mg/kg/day dose groups (no significance in the 50 mg/kg/day group)
  - Uterus – wet (g) was 0.4201, 0.3166, 0.3398, 0.2170g across the dose groups.
  - Uterus – blotted (g) was 0.3371, 0.2761, 0.2863, 0.1988 g across the dose groups.
- Statistically significant reduction ($p<0.05$) in mean absolute and relative spleen weights in the 200 mg/kg/day groups
  - 0.4681, 0.4814, 0.4170, 0.3917g across dose groups
- Statistically significant reduction ($p<0.05$, $p<0.01$) in mean absolute and relative thymus weights at 200 mg/kg/day
  - 0.5948, 0.5931, 0.5735, 0.5127g across dose groups
- Reduction in luminal fluid in all dose groups (no statistical significance)
  - 0.0829, 0.0405, 0.0535, 0.0182g across dose groups

Histological Effects

- Decreased corpora lutea in the ovaries of the 200 mg/kg/day group animals
  - 0/15 versus 3/15observations (0, 200 mg/kg/day)
- Absent corpora lutea in the ovaries of the 200 mg/kg/day group animals
  - 0/15, 1/15observations (0, 200 mg/kg/day)
- Uterine atrophy at 200 mg/kg/day
  - 0/15, 8/15observations (0, 200mg/kg/day)

Other Findings

Eleven females in the 800 mg/kg/day group were found dead at 24-28 days of age as a result of test article administration. All females were found dead on the morning after the previous daily dose.

Discussion and Conclusions

Female Reproductive Toxicity

Based on the results of this study, TPP (distilled) 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 by earlier age at the first occurrence of estrus. Mean absolute and/or relative (to final body weight) wet and blotted uterus weights (and thus, luminal fluid weight), ovary/oviduct, spleen weights and thymus gland weights in the 200 mg/kg/day groups were lower than the control group values.

Animal deaths attributed to the 800 mg/kg/day dose level in this study, were a result of elevated test article administration levels.

Although lower mean absolute ovary/oviduct weights and wet and/or blotted uterus weights did not occur in a dose-related manner in the 10 and 50 mg/kg/day groups, the reductions in these weights were considered test article-related. No test article-related effects on mean serum E_2, LH, T_4 or TSH levels were observed at 10, 50 and 200 mg/kg/day.

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

Oral gavage administration of TPP (distilled) to juvenile female rats resulted in acceleration of vaginal patency, a marker of sexual maturation, and earlier age at the first occurrence of estrus at 50 and 200 mg/kg/day.

Female Pubertal Assay (Knapp, 2007a)

Study Design

Test material, in the vehicle, corn oil, was administered orally by gavage once daily for 20 consecutive days to three groups each of 15 Crl:CD (SD) immature female rats. Dosage levels were 5, 20 and 60 mg/kg/day. The dose volume for all groups 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)	20
Ovary Weight (Female)	5

Results

Body Weight Changes

- No test article-related changes in mean body weights or body weight gains were observed.

Vaginal Opening

- Accelerated (early) vaginal opening (VO) observed at 60 mg/kg/day group
  - Mean day of attainment of vaginal opening was 33.2, 33.3, 32.7, and 29.1 days across the dose groups

  Note: acceleration was not observed in F1 offspring in the one-generation reproduction gavage study at 25 mg/kg/day; exposure post-weaning (intentionally not conducted in this 1-gen) may be necessary to alter puberty timing.
- Decreased body weight at vaginal opening at 60 mg/kg/day group ($p<0.01$)
  - Body weight (g) was 110.9, 108.2, 109.5, and 89.2 g at attainment across the dose groups

Histological Effects

- Decreased corpora lutea in the ovaries of the 20 and 60 mg/kg/day group animals
  - 1/15, 1/15, 3/15, and 4/15 observations across dose groups (0, 5, 20, 60 mg/kg/day)
- Uterine hypoplasia at 60 mg/kg/day
  - 2/15, 0/15, 0/15, 7/15 observations across dose groups
- Thyroid gland follicular hypertrophy at 20 and 60 mg/kg/day groups

- 1/15, 1/15, 3/15, and 10/15

Note: In rats, changes in thyroid gland may occur early in repeat-exposure studies and resolve with longer exposure (e.g. may exist at 14-days but might not be observed at the end of 28 or 90 day studies).

Hormone Effects

- There were no changes to TSH or T4 levels in this study; no other serum hormone levels were measured

Discussion and Conclusions

Repeated Dose Toxicity

There were no findings of generalized systemic toxicity.

Female Reproductive Toxicity

Oral gavage administration of TPP to immature female rats resulted in acceleration of vaginal patency, a marker of sexual maturation, at 60 mg/kg/day, the highest dose tested (29.1 days vs. 33.2 days in the control group). As TPP administration did not affect body weight, there was also a significant reduction in body weight at attainment (89 g vs. 111 g in the control group). There were no changes in organ weights (liver, kidneys, adrenal glands, uterus, ovaries, pituitary, thyroid).

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

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

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

Female Pubertal Assay (Knapp, 2007b)

Study Design

Test material in the vehicle, corn oil, was administered orally by gavage once daily for 20 consecutive days to three groups each of 15 Crl:CD (SD) IGS BR immature female rats. Dosage levels were 60, 250 and 1000 mg/kg/day, and the dose 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)	< 60
Ovary Weight (Female)	< 60

Results

Two females of the 1000 mg/kg/day dosage group were found dead following two days of dosing. There were no internal findings to clarify the cause of these deaths.

Body Weight Changes

- Reductions in mean body weight gains at 1000 mg/kg/day ($p<0.05$ or $p<0.01$) occurred during the first three dosing intervals (PND 22-25). However, when the entire treatment period (PND 22-42) was evaluated, mean body weight gain in the 1000 mg/kg/day TS03018 group was similar to that in the control group.

- No TS03018-related effects on mean body weights or body weight gains were observed in the 60 and 250 mg/kg/day groups.

Vaginal Opening

- Accelerated (early) vaginal opening (VO) observed at 60, 250, and 1000 mg/kg/day ($p<0.01$).
  - Mean day of attainment of vaginal opening was 34.5, 28.3, 27.9, 27.6 days across the dose groups
- Decreased body weight at vaginal opening at 60 , 250, and 1000 mg/kg/day
  - Body weight (g) was 105.9, 75.4, 75.2, and 67.4 g at attainment across the dose groups

Estrous Cycle

- Accelerated (early) vaginal opening (VO) observed at 60, 250, and 1000 mg/kg/day

  - Mean first occurrences of estrus in all treatment groups were earlier than in the control group. The differences were significant ($p<0.05$ or $p<0.01$) in the 60 and 1000 mg/kg/day TS03018 groups; all differences were consistent with an estrogenic effects of the test article.

    Note: No differences in mean estrous cycle length were observed when comparing the TS03018-treated groups to the control group. Estrous cycle lengths in females of this age are highly variable, and combined with the limited number of animals and days of evaluation, evidence of a test article-related effect was inconclusive.

Histological Effects

Not studied.

Organ Weights

- Statistically significant reduction ($p<0.05$ or $p<0.01$) in mean absolute ovary weights
  - Ovary weight (g) without oviducts was 0.037, 0.030, 0.034, and 0.021 g across the dose groups
  - Ovary weight (g) with oviducts was 0.059, 0.049, 0.046, and 0.030 g across the dose groups
- Reduction in mean absolute and relative uterus (wet and blotted) weights in the 250 and 1000 mg/kg/day groups
  - Uterus – wet (g) was 0.217, 0.278, 0.172, and 0.152 g across the dose groups.
  - Uterus – blotted (g) was 0.190, 0.218, 0.151, and 0.142 g across the dose groups

  Note: The reductions in mean uterine weights were attributed to the test article; however, they were not considered indicative of estrogen modulation.
- Increase in mean absolute and relative liver weight in the 250 and 1000 mg/kg/day groups
  - 4.964, 5.124, 5.518, and 7.394 g across dose groups

- Statistically significant increase ($p<0.05$ or $p<0.01$) in mean absolute and relative adrenal gland weights in the 60, 250 and 1000 mg/kg/day groups

  - 0.023, 0.026, 0.029, and 0.031g across dose groups

Hormone Effects

Not studied.

Other Findings

Two females in the 1000 mg/kg/day group were found dead at 24 days of age, following two doses. These animals had no clinical findings prior to death. There were no internal findings for these females at necropsy. These mortalities were potentially test article-related.

Discussion and Conclusions

Repeated Dose Toxicity

There were no findings of generalized systemic toxicity.

Female Reproductive Toxicity

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

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

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

Study Design

The objective of this study was to evaluate the ability of the test substance, TPP, to inhibit the binding of a radiolabeled ligand, ^{3}H-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 ^{3}H-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 % ^{3}H-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

As suggested by NIH Publication No. 03-4506, the potential for variation in IC_{50} 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 IC_{50} 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.

In Vitro Rat Uterine Estrogen Receptor Competitive Binding Assay (Thomas *et al.*, 2012b)

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 (Raleigh, NC). Each animal was ovariectomized approximately 9 days before euthanasia. 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 24-hour incubation of the ligand with the receptor, the specific binding of the radiolabeled 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 ^{3}H-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 radioligand 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 radioligand is displaced (IC_{50}) 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 uterinecytosoll 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 IC_{50} 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 IC_{50} values for test and reference compounds:

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

The %RBA observed for all test chemicals exhibited the following order:

[un-labeled Estradiol > 19-Norethindrone > Test Substance TPP]

The arithmetic mean IC_{50} for the reference estrogen was found to be 1.2 nM. This is close to the expected IC_{50} of 1.4 nM, as calculated by summing the concentration of radioligand (1 nM) and its affinity for the ER (K_d, 0.41 nM). The arithmetic calculation of the mean IC_{50} 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 IC_{50} for 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 0.11%.

Reproductive Toxicity Studies of TPP-Derived Substances (Supporting Studies)

The test materials in these studies are TPP-derived substances that contain TPP as an impurity. These studies validate the SCL for TPP.

Table 29: Overview of TPP derivative studies (substances that contain TPP as impurity)

Test Material EC No.	Method	Results	Remarks	Reference
272-234-3 (6.7 wt% TPP)	Rat (Sprague-Dawley) male/female two-generation study	NOAEL (Parental toxicity) (F0 and F1): 50 mg/kg/day (male/female) based on test mat. (3.4 mg TPP/kg/day)(clinical signs, inhibition of body weight	1 (reliable without restriction) Supporting	Nemec *et al.* (1995)

Test Material EC No.	Method	Results	Remarks	Reference
	Oral: gavage 0, 50, 300 & 1000 mg/kg/day (0, 3.4, 20.1, 67 mg/kg/day TPP) (actual ingested) Note: An additional satellite cross-breeding study was conducted using a separate group of F1 animals. High-dose males were mated to control females while high-dose females were mated to control males to investigate if observed effects were specific to one sex. The satellite F1 groups (F1-satellite) were dosed beginning on PND 22 for a minimum of 88 days prior to mating, then throughout breeding, gestation and lactation OECD Guideline 416 (Two-Generation Reproduction Toxicity Study)	gain, increased pituitary gland and liver weights and decreased testes, epididymides and ovary weights.) NOAEL (Reproductive toxicity) (F0 and F1): 300 mg/kg/day (male/female) (20.1 mg TPP/kg/day) based on test material. (Based on reduced fertility indices, apparent dystocia and reduced live litter size) NOAEL (Neonatal toxicity) (F1 and F2): 50 mg/kg/day based on test mat. (3.4 mg TPP/kg/day) (Increased number of dead pups on lactation day 0 and reduced pup body weights. Equivocal neonatal toxicity was observed at the 300 mg/kg/day dose level by an increased number of dead F1 pups on lactation day 0.)	study Experimental result	
415-930-6 (3.8 wt% TPP)	Rat (Sprague-Dawley) male/female Two-generation study Oral: gavage 0, 50, 250 & 1000 mg/kg/day (0, 1.9, 9.5, 38 mg/kg/day TPP) (actual ingested) F0 generation was dosed for 3 weeks after weaning of the F0a litters, at which time a second phase of mating was initiated. F0b mating-phase animals were again paired to dose-matched mates and all animals continued dosing through the F0b mating and gestation periods. A separate collection of pups were selected from the F0a litters that were outside the mean weaning dates for observation of	NOAEL (adults and offspring) 50 mg/kg based on test material (1.9 mg TPP/kg/day) (body weight, reduced litter size, pregnancy rate, male sexual maturation delay)	1 (reliable without restriction) Supporting study Experimental result	Wood *et al.* (2002)

Test Material EC No.	Method	Results	Remarks	Reference
	sexual maturation only (F1-select). OECD Guideline 416 (Two-Generation Reproduction Toxicity Study)			
430-180-1 (26 wt% TPP)	Rat (Sprague-Dawley) male/female Two-generation study Oral: gavage 0, 5, 30 & 150 mg/kg/day (0, 1.3, 7.8, 39 mg/kg/day TPP) (actual ingested) Exposure: (F0) dosed for a minimum of 10 weeks prior to mating and then to dose-matched mating pairs throughout the mating, gestation and lactation phases. (F1) offspring from each dose group were selected to proceed into the main study (at weaning). (F1) dosed for a minimum of 10 weeks Following this dosing period, the main study F1 animals were paired within dosing groups and subsequently dosed throughout mating, gestation and lactation to yield the F2 litters. OECD Guideline 416 (Two-Generation Reproduction Toxicity Study)	NOAEL (fertility and reproductive performance) 150 mg/kg based on test material (39 mg TPP/kg/day) (male body weight gain, accelerated female sexual maturation, reduced seminal vesicle/coagulating gland tissue weights, increased homogenization- resistant spermatid)	1 (reliable without restriction) Supporting study Experimental result	Wood *et al.* (2003)
455-880-2 (2.5 wt% TPP)	Rat (Sprague-Dawley) One-generation study Oral: gavage 0, 50, 170, and 500 mg/kg/day (0, 1.25, 4.25, and 12.5 mg/kg/day TPP) (actual ingested) Exposure: 70 consecutive days (F0: Male & Female) Dosing for the F0 males	NOAEL (reproductive): 500 mg/kg/day (12.5 mg TPP/kg/day) NOAEL (systemic): 170 mg/kg/day (hematology) (4.25 mg TPP/kg/day) NOAEL (neonatal): 500 mg/kg/day (12.5 mg TPP/kg/day)	1 (reliable without restriction) Supporting study Experimental result	Knapp *et al.*(2008)

Test Material EC No.	Method	Results	Remarks	Reference
	continued throughout mating and through the day prior to euthanasia, for a total of 133 to 139 doses. The F0 females continued to be dosed throughout mating, gestation and lactation, through the day prior to euthanasia, for a total of 128 to 133 doses. (Once daily) Additional endpoints evaluated: hematology, serum chemistry, spermatogenic evaluation, estrous cyclicity, OECD Guideline 415 (One-Generation Reproduction Toxicity Study)			
272-234-3 (6.7 wt% TPP)	Rat (Sprague-Dawley) 90 day study(Male & Female) Oral: gavage 125, 250, 500, 1000 mg/kg/day (8.4, 16.7, 33.4, 67 mg/kg/day TPP) (actual ingested) OECD Guideline 408 (Repeated Dose 90-day Oral Toxicity Study in Rodents)	NOAEL: 1000 mg/kg bw/day (67 mg TPP/kg/day) (actual dose received) based on: test mat.	1 (reliable without restriction) Supporting study Experimental result	Haas *et al*. (2010)

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

Study Design

A multi-generational study designed to meet or exceed the OECD 416 Guideline (1983) evaluated the potential of EC No.272-234-3 in a peanut oil suspension to cause adverse reproductive effects at doses of 0 (peanut oil vehicle), 50, 300 or 1000 mg/kg/day when administered once daily by gavage (at dosage volume of 5 ml/kg) to male and female Sprague-Dawley Crl:CD®BR rats (30 males and 30 females per group). Both sexes of the parental generation (F0) were treated for at least 71 consecutive days prior to mating in dose-matched pairs, and throughout breeding, gestation and lactation until necropsy. Estrous cyclicity and semen analysis were not included in the study design, as they were not specified in the existing test guidelines available at that time. F0 offspring (the F1 generation) were potentially exposed to test article *in utero* and through lactation during postnatal days (PND) 0 – 21. F1 animals selected for breeding were administered test article beginning on PND 22 for a minimum of 77 days prior to mating in dose-matched pairs, and then throughout breeding, gestation and lactation. All litters of the primary study groups (F0 and F1-main offspring)

were necropsied on postnatal day PND 21. Due to adverse reproductive effects observed in the F0 generation, an additional satellite cross-breeding study was conducted using a separate group of F1 animals. High-dose males were mated to control females while high-dose females were mated to control males to investigate if observed effects were specific to one sex. The satellite F1 groups (F1-satellite) were dosed beginning on PND 22 for a minimum of 88 days prior to mating, then throughout breeding, gestation and lactation. F1-satellite females were allowed to deliver and rear F2-satellite offspring to lactation day 7 at which point dams and pups were sacrificed and necropsied. F1-satellite males were retained for an additional 33 day recovery period then sacrificed and necropsied.

EC No.272-234-3 contains approximately 6.7 wt% residual tetrapropenyl phenol. The converted approximate equivalent residual concentration TPP present in each dose level of 0, 50, 300 and 1000 mg/kg/day is (0), (3.4), (20.1) and (67) mg/kg/day respectively. Where presented, TPP dose appears in parenthesis to distinguish TPP dose from test article dose.

Summary of No Observed Adverse Effect Levels

End Point/Generation	NOAEL (mg/kg bw/day)
Parental Toxicity (F0 & F1)	50 (3.4 mg TPP/kg bw/day)
Reproductive Toxicity (F0 & F1)	300 (20.1 mg TPP/kg bw/day)
Neonatal Toxicity (F1 & F2)	50 (3.4 mg TPP/kg bw/day)

Clinical Observations

- There were no treatment related deaths.
- Salivation and discoloration noted 1 hour post-dosing at 1000 and 300 mg/kg/day test material (67 and 20.1 mg/kg/day TPP, respectively) in F0 animals of both sexes.
- Increased incidence of red discharge from the vaginal opening noted at 1 hour post-dosing in F0 females treated at 1000 mg/kg/day (67 mg/kg/day).

  Note: The study director did not make a direct assertion that the findings noted above are test material related but notes obliquely that no findings that could be attributed to test material were noted at the lower dose. Additionally, the study director does not address whether these findings were considered adverse. It is our interpretation that red discharge from the vaginal opening is adverse.

Body Weight

- F0 generation
  - Males dosed at 1000 mg/kg/day (67 mg/kg/day TPP)
    - F0 males had reduced body weight beginning at week 3 through necropsy
    - F0 males were observed to have reduced body weight gain throughout the majority of the study

- Females dosed at 1000 mg/kg/day (67 mg/kg/day TPP)
  - F0 females had transiently reduced body weight gain initially (week 0 – 1) and then, during weeks 7 – 8, but otherwise were similar to control throughout the study
  - Mean body weight of F0 females was significantly reduced compared to controls on gestation day 20 but not at any other time
  - Mean body weight gain of F0 females was significantly reduced compared to controls on gestation days 14 – 20. Mean body weight gain in this group was also reduced compared to controls during gestation days 7 – 11 and 11 – 14 although the differences were not statistically significant
- All dosed at 300 mg/kg/day (20.1 mg/kg/day TPP)
  - F0 males had decreased body weight beginning at week 14 through necropsy
  - F0 males of this group had transiently reduced body weight gain at various points throughout the study (weeks 3 – 4, 7 – 10 and 18 – 19)
  - F0 females also had transiently reduced body weight gain initially (week 0 – 1) and then during weeks 7 – 8 but otherwise were similar to control throughout the study

- F1 generation main study
  - Males dosed at 1000 mg/kg/day (67 mg/kg/day TPP)
    - Significantly reduced body weight compared to controls (564 ± 57.6 vs. 651 ± 60.9 g, treated vs. control)
    - F1-main males had reduced body weight beginning at week 20 through necropsy
    - F1-main males were observed to have reduced body weight gain throughout the majority of the study
  - Females dosed at 1000 mg/kg/day (67 mg/kg/day TPP)
    - F1-main females had significantly reduced body weight compared to control values during weeks 20 -29
    - F1-main females mean body weight gain was significantly reduced, compared to controls on Gestation days 14 – 20. Mean body weight gain in this group was also reduced, compared to controls during gestation days 7 – 11 and 11 – 14, although the difference was not statistically significant
  - All dosed at 300 and 50 mg/kg/day (20.1 and 3.4 mg/kg/day TPP, respectively)
    - Any differences observed were attributed to biological variability by the study director
  - Feed consumption was not changed consistently in males or females in any dose group

<u>Hematology:</u>

Not evaluated as part of this study

<u>Serum Chemistry:</u>

Not evaluated as part of this study

Organ Weights

F0 Generation

- F0 males treated at 1000 mg/kg/day (67mg/kg/day TPP):
  - Increased mean absolute kidney weight (4.63 ± 0.599 vs. 4.14 ± 0.45, approximately + 12%)
  - Decreased mean absolute epididymides weight (1.35 ± 0.143 vs. 1.47 ± 0.115, treated vs. control, approximately -9.1 %)
  - Increased mean absolute pituitary weight (0.0192 ± 0.00284 vs. 0.0157 ± 0.00227, approximately + 22%)
  - Increased mean brain weight relative to body weight (0.423 ± 0.0352 vs. 0.370 ± 0.0516, treated vs. control, approximately + 14%)*
  - Increased mean liver weight relative to body weight (4.931 ± 0.5608 vs. 4.138 ± 0.4869, treated vs. control, approximately +19%)*
  - Increased mean kidney weight relative to body weight (0.900 ± 0.0984 vs. 0.696 ± 0.070, treated vs. control, approximately +29%)*
  - Increased mean testes weight relative to body weight (0.694 ± 0.0700 vs. 0.615 ± 0.0757, treated vs. control, approximately +13%)*

    Increased mean pituitary weight relative to body weight (0.004 ± 0.0006 vs. 0.0027 ± 0.0005, treated vs. control, approximately +48%)*
- F0 males treated at 300 mg/kg/day (20.1 mg/kg/day TPP):
  - Increased mean kidney weight relative to body weight (0.785 ± 0.0642 vs. 0.696 ± 0.070, treated vs. control approximately +13%)*
  - Increased mean testes weight relative to body weight (0.658 ± 0.0547 vs. 0.615 ± 0.0757, treated vs. control, approximately +7%)*
  - Increased mean pituitary weight relative to body weight (0.0032 ± 0.0005 vs. 0.0027 ± 0.0005, treated vs. control, approximately +18%)*

    **NOTE: Identified organ weight changes relative to body weight corresponded to significantly reduced final body weight compared to controls.*

    F0 male 1000 mg/kg/day final body weight – 515 ± 43.0, ~ -14% vs. control

    F0 male 300 mg/kg/day final body weight – 558 ± 39.4, ~ - 7% vs. control

    F0 male control group final body weight – 600 ± 81.5
- F0 females treated at 1000 mg/kg/day (67 mg/kg/day TPP):
  - Increased mean absolute liver weight (15.23 ± 2.394 vs. 13.15 ± 1.615, treated vs. control, approximately + 16%)

- Decreased mean absolute ovary weights (0.1221 ± 0.02588 vs. 0.1508 ± 0.02119, treated vs. control, approximately - 19%)
- Increased mean liver weight relative to body weight (5.124 ± 0.4474 vs. 4.229 ± 0.2798, treated vs. control, approximately + 17%)
- Decreased mean ovary weights relative to body weight (0.042 ± 0.0098 vs. 0.049 ± 0.0071, treated vs. control, approximately - 16%)
- Decreased mean pituitary weight relative to body weight (0.007 ± 0.0010 vs. 0.006 ± 0.0013, treated vs. control, approximately - 16%)

F1 Generation main study

- F1 males treated at 1000 mg/kg/day (67mg/kg/day TPP):
  - Decreased mean absolute liver weight (22.86 ± 2.725 vs. 25.92 ± 4.932, treated vs. control, approximately - 12%)
  - Decreased mean absolute epididymides weight (1.25 ± 0.161 vs. 1.50 ± 0.139, treated vs. control, approximately - 17 %)
  - Decreased mean absolute testes weight (3.52 ± 0.564 vs. 3.82 ± 0.393, treated vs. control, approximately - 8 %)
  - Increased mean absolute pituitary weight (0.0193 ± 0.00262 vs. 0.0153 ± 0.00276, approximately + 26%)
  - Increased mean brain weight relative to body weight (0.439 ± 0.0367 vs. 0.348 ± 0.0354, treated vs. control, approximately + 24%)*
  - Increased mean liver weight relative to body weight (4.608 ± 0.3278 vs. 4.113 ± 0.4859, treated vs. control, approximately + 12%)*
  - Increased mean kidney weight relative to body weight (0.895 ± 0.01127 vs. 0.669 ± 0.0487, treated vs. control, approximately + 27%)*
  - Increased mean testes weight relative to body weight (0.715 ± 0.1249 vs. 0.614 ± 0.0810, treated vs. control, approximately + 16%)*
  - Increased mean pituitary weight relative to body weight (0.004 ± 0.0008 vs. 0.002 ± 0.0006, treated vs. control)

    **NOTE: Identified organ weight changes relative to body weight corresponded to significantly reduced final body weight compared to controls.*

    F0 male 1000 mg/kg/day final body weight – 496 ± 47.3, ~ -21% vs. control

    F0 male 300 mg/kg/day final body weight – 550 ± 66.4, ~ - 12% vs. control

    F0 male control group final body weight – 627 ± 65.7
- F1 males treated at 300 mg/kg/day (20.1 mg/kg/day TPP):
  - Decreased mean absolute liver weight (22.50 ± 3.913 vs. 25.92 ± 4.932, treated vs. control, approximately - 13%)

- Decreased mean absolute epididymides weight (1.25 ± 0.161 vs. 1.50 ± 0.139, treated vs. control, approximately - 17 %)
- Increased mean brain weight relative to body weight (0.404 ± 0.0452 vs. 0.348 ± 0.0354, treated vs. control, approximately + 16%)*
- Increased mean kidney weight relative to body weight (0.784 ± 0.01127 vs. 0.669 ± 0.0487, treated vs. control, approximately + 17%)*
- Increased mean testes weight relative to body weight (0.695 ± 0.0673 vs. 0.614 ± 0.0810, treated vs. control, approximately + 13%)*
- Increased mean pituitary weight relative to body weight (0.003 ± 0.0005 vs. 0.002 ± 0.0006, treated vs. control)

o F1 females treated at 1000 mg/kg/day (67 mg/kg/day TPP):

- Increased mean absolute liver weight (13.66 ± 1.394 vs. 12.35 ± 2.189, treated vs. control, approximately + 11%)
- Increased mean absolute pituitary weight (0.0190 ± 0.00254 vs. 0.0158 ± 0.00231, treated vs. control, approximately - 20%)*
- Increased mean brain weight relative to body weight (0.692 ± 0.0757 vs. 0.627 ± 0.0526, treated vs. control, approximately + 10%)*
- Increased mean liver weight relative to body weight (4.703 ± 0.4702 vs. 3.906 ± 0.5545, treated vs. control, approximately + 20%)*
- Increased mean kidney weight relative to body weight (0.815 ± 0.0854 vs. 0.734 ± 0.0571, treated vs. control, approximately + 11%)*
- Increased mean pituitary weight relative to body weight (0.007 ± 0.0012 vs. 0.005 ± 0.0008, treated vs. control, + 40%)*

  **NOTE: Identified organ weight changes relative to body weight corresponded to significantly reduced final body weight compared to controls.*

  F1-main female final body weight – 292 ± 32.0, ~ -28% vs. control

  F1-main female control group final body weight – 316 ± 24.3

o F1 females treated at 300 mg/kg/day (20.1 mg/kg/day TPP)

- Increased mean brain weight relative to body weight (0.665 ± 0.0580 vs. 0.627 ± 0.0526, treated vs. control, approximately + 10%)*
- Increased mean liver weight relative to body weight (4.252 ± 0.3218 vs. 3.906 ± 0.5545, treated vs. control, approximately + 9%)*

  **NOTE: Identified organ weight changes relative to body weight corresponded to significantly reduced final body weight compared to controls.*

  F1-main female final body weight – 292 ± 32.0, ~ -28% vs. control

  F1-main female control group final body weight – 316 ± 24.3

- F1-satellite males treated at 1000 mg/kg/day (67 mg/kg/day TPP:
  - Decreased mean absolute testes weight (3.77 ± 0.282 vs. 3.92 ± 0.269, treated vs. control, approximately - 4%)
  - Decreased mean absolute epididymides weight (1.34 ± 0.123 vs. 1.48 ± 0.138, treated vs. control, approximately - 9 %)
  - Increased mean absolute pituitary weight (0.162 ± 0.00217 vs. 0.138 ± 0.00205, treated vs. control, approximately + 17 %)
  - Decreased mean testes weight relative to body weight (0.673 ± 0.0746 vs. 0.606 ± 0.0668, treated vs. control, approximately + 11%)
  - Increased mean pituitary weight relative to body weight (0.003± 0.0003 vs. 0.002 ± 0.0004, treated vs. control)

Histological Changes

- F0 generation - no histological changes were considered treatment related in any dose group
- F1 generation - no histological changes were considered treatment related in any dose group
- F2 generation - no histological changes were considered treatment related in any dose group

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 frequency of plugs noted in the highest dose group. The biological significance of this finding is not known.

Reproductive performance and fertility

Estrous Cyclicity

Not evaluated as part of the study (not an endpoint within OECD 416 [1983]).

Semen Analysis

Not evaluated as part of the study (not an endpoint within OECD 416 [1983]).

Fertility

- F0 generation
  - Dosed at 1000 mg/kg/day (67 mg/kg/day TPP)
    - Fertility index adversely affected (73.3% vs. 96.7%, treated vs. control)
    - Eight males failed to sire a litter
    - Seven females had evidence of mating but failed to produce a litter
    - The pre-coital interval was significantly increased compared to control (4.4 days vs., 2.4 days, treated vs. control). This increase was due to three females with unusually long pre-coital periods (11 – 13 days), not a general trend in the test population.
  - Dosed at 300 mg/kg/day (20.1 mg/kg/day TPP)

        - All observations were similar to control
- F1-main generation
    - Dosed at 1000 mg/kg/day (67 mg/kg/day TPP)
        - Fertility index adversely affected (76.7% vs. 93.3%, treated vs. control)
        - No effect on pre-coital interval as noted
    - Dosed at 300 and 50 mg/kg/day (20.1 and 3.4 mg/kg/day TPP)
        - No effects on fertility or pre-coital interval were observed in the either dose group
- F1-satellite generation
    - Females dosed at 1000 mg/kg/day (67 mg/kg/day TPP)
        - Mating index not affected by treatment (100%)
        - Fertility index adversely affected (55.2%)
        - No effect on pre-coital interval as noted
        - 15 females had evidence of mating but did not deliver a litter
        - 13 females were non-gravid
    - Females dosed at 0 mg/kg/day (0 mg/kg/day TPP equivalent dose)
        - Mating index not affected by treatment (96.7%)
        - Fertility index comparable to historic control (96.7 %)
        - No effect on pre-coital interval as noted

<u>Gestation</u>

- F0 generation dosed at 1000 mg/kg/day (67 mg/kg/day TPP)
    - No effect on length of gestation
    - No instances of dystocia were noted at any dose
- F1-main generation dosed at 1000 mg/kg/day (67 mg/kg/day TPP equivalent dose)
    - No effect on length of gestation
    - Three females found dead with findings consistent with dystocia
    - One female sacrificed in extremis with findings consistent with dystocia
- F1-satellite females all doses
    - Gestation length was comparable to historical controls
    - No instances of dystocia were noted

<u>Female Reproductive Toxicity</u>

- F0 generation
  - Dosed at 1000 mg/kg/day (67 mg/kg/day TPP)
    - Fertility index adversely affected (73.3% vs. 96.7%, treated vs. control)
    - Seven females had evidence of mating but did not deliver a litter
    - Mean live litter size significantly reduced compared to control (8.8 vs. 12.6 pups, treated vs. control)
    - Number pups born dead significantly increased compared to control (19 dead of 187 total pups born vs. 3, treated vs. control)
    - Mean pup body weights significantly reduced compared to control at postnatal days 14, 21 and 28
      - 14 day pup mean body weight – 28.6 ± 3.89 vs. 31.5 ± 3.11, ~ -9 % vs. control
      - 21 day pup mean body weight – 43.7 ± 5.54 vs. 49.1 ± 4.28, ~ -11% vs. control
      - 28 day pup mean body weight – 75.2 ± 9.12 vs. 80.7 ± 5.59, ~ -7 % vs. control
  - Dosed at 300 and 50 mg/kg/day (20.1 and 3.4 mg/kg/day TPP, respectively)
    - Fertility index not adversely affected (93.3% and 93.3 % vs. 96.7%, 300 and 50 mg/kg/day vs. control, respectively)
    - Mean live litter size comparable to control at both dose levels
    - Number pups born dead significantly increased compared to control (26 dead of 372 total pups born vs. 3, treated vs. control)
    - Mean pup body weights comparable to control
- F1-main generation (main study)
  - Dosed at 1000 mg/kg/day (67 mg/kg/day TPP)
    - Fertility index adversely affected (76.7% vs. 93.3%, treated vs. control)
    - Twelve females had evidence of mating but did not deliver a litter
    - Group mean live litter size was reduced compared to control (12.3 ± 2.9 vs. 14.4 ± 2.4 born)
    - Group mean live litter size was reduced compared to control on Day 1 post-partum (12.2 ± 3.0 vs. 14.2 ± 2.6 born)
  - Dosed at 300 and 50 mg/kg/day (20.1 and 3.4 mg/kg/day TPP, respectively)
    - No difference in total corpora lutea compared to control

  Note: Increased number of dead pups of the F1 generation dosed at 300 mg/kg/day dose group (20.1 mg/kg/day TPP) was considered of equivocal biological significance because the effect was not repeated in the F2 generation at the same dose.

- F1-satellite generation (cross-breeding study)
    - Dosed at 1000 mg/kg/day (67 mg/kg/day TPP) paired with untreated males
        - Fertility index adversely affected (55.2%, 16/29)
        - The proportion of pups found dead to total pups born was increased (9/79, 11.4%)
        - Mean live litter size was reduced (5.8 pups, no statistical analysis performed)

Male Reproductive Toxicity

- F0 generation
    - No significant effect on mating indices occurred at any dose level
    - Males that did not sire a litter - no significant effect on concentration, motility or morphology of sperm
        - Dosed at 1000 mg/kg/day (67 mg/kg/day TPP)
            - Fertility index significantly reduced compared to control (73.3% vs. 96.7%, treated vs. control)
            - Mating index reduced but not statistically significant and within historic control range (86.7% vs. 96.7%, treated vs. control)
            - 8 males did not sire a litter at this dose
        - Dosed at 300 and 50 mg/kg/day (20.1 and 3.4 mg/kg/day TPP, respectively)
            - Fertility indices not adversely affected (93.3% and 93.3 % vs. 96.7%, 300 and 50 mg/kg/day vs. control, respectively)
- F1-main generation (main study)
    - No significant effect on mating indices occurred at any dose level
    - Males that did not sire a litter - no significant effect on concentration, motility or morphology of sperm
        - Dosed at 1000 mg/kg/day (67 mg/kg/day TPP)
            - Fertility index adversely affected (76.7% vs. 90.0%, treated vs. control)
            - Mating index reduced but not statistically significant and within historic control range (86.7% vs. 96.7%, treated vs. control)
            - Seven males did not sire a litter at this dose
- F1-satellite generation (cross-breeding study)
    - No significant effect on mating or fertility indices observed in either treatment group
        - Mating index
            - 1000 mg/kg/day (67 mg/kg/day TPP) – 89.3%

- Control males (0 mg/kg/day) – 93.3%

- Fertility index
  - 1000 mg/kg/day (67 mg/kg/day TPP) - 89.3% (25/28)
  - Control males (0 mg/kg/day) – 90.0%

Discussion and Conclusions

EC No. 272-234-3 dosed at 1000 mg/kg/day (67 mg/kg/day TPP) was associated with general reduction in body weight and body weight gain in males of all three study groups but only transiently in females at different points throughout the studies. Some changes in food consumption were noted intermittently but were not associated with adverse biological effects due to test material administration.

Decreased fertility indices were noted in F0 and F1 generation males and females dosed at 1000 mg/kg/day (67 mg/kg/day TPP). In the satellite breeding study, fertility indices were reduced for the high-dose females mated to control males, but not the high-dose males mated to control females. This suggests that female reproductive function appeared to be more affected by test material administration than male reproductive function at this dose.

An increased number of males in the F0 and F1-main groups treated at 1000 mg/kg/day (67 mg/kg/day TPP) failed to sire litters (8/30 and7/28, F0 and F1-main, respectively), compared to controls (1/30 and 3/30, F0 and F1-main generations respectively), although only 3/28 males dosed at 1000 mg/kg/day (67 mg/kg/day TPP) in the satellite cross-breeding study did not sire litters. An increased number of females in the F0 and both F1 study groups treated at 1000 mg/kg/day (67 mg/kg/day TPP) with evidence of mating failed to deliver litters (7/29, 12/29 and15/29, F0, F1-main and F1-satellite, respectively), compared to F0 and F1-main controls (0/29 and 2/29, respectively). Dystocia occurred in 5 F1-main study females, but was not observed in the F0 or F1-satellite study groups.

Macroscopic findings included altered tissue organ weights. Epididymides and ovary weights were reduced in males and females, respectively, of both generations dosed at 1000 mg/kg/day (67 mg/kg/day TPP). Pituitary gland weights were increased for the 1000 mg/kg/day group males (F_0, F_1, F_1 satellite) and females (F_0 and F_1). No histological findings in reproductive tissues were noted in males or females of either generation. Semen parameters were evaluated for males that failed to sire a litter, but no effects were noted in either generation in any parameter evaluated.

A reduction in live litter size at birth was observed in F0 and F1 animals treated at 1000 mg/kg/day (67 mg/kg/day TPP) and an increased number of pups were born dead in both generations. No difference in pup viability was observed at any dose in either generation after postnatal day 1.

While a limited number of additional statistically significant tissue effects were observed in discrete subsets of the tested population, no organ weight changes were correlated with histological findings in any instance.

Two Generation Oral (Gavage) Reproductive Toxicity Study in the Rat (Wood *et al.*, 2002)

Study Design

A study originally designed to meet or exceed the OECD 415 (1983) evaluated the effects of the test material (EC No. 415-930-6) on growth and reproductive performance in rat (Sprague-Dawley Crl:DC IGS BR). Because of findings from the initial mating phase (F0a), a second mating phase was included (F0b). A second generation was also added using offspring from the F0a mating phase. These modifications to the protocol resulted in a final study that meets or exceeds the OECD 416 Guideline.

Test material was administered orally by gavage to the F0 generation (28 males and 28 females per group) at doses of 0, 50, 250 or 1000 mg/kg/day in a corn oil suspension (dose volume of 5 ml/kg) for at least 10 weeks prior to mating and then to dose-matched mating pairs throughout the mating, gestation and lactation phases. Because of equivocal findings from the F0a mating phase, the F0 generation was dosed for three weeks after weaning of the F0a litters, at which time a second phase of mating was initiated. F0b mating-phase animals were again paired to dose-matched mates and all animals continued dosing through the F0b mating and gestation periods. Care was taken to avoid re-pairing of breeding mates from the F0a phase. F0 females and the F0b generation were terminated on Day 20 of gestation.

At weaning, offspring from each dose group of the F0a mating were selected to form the F1-main generation and were dosed through postweaning maturation, mating, gestation and lactation. A separate collection of pups were selected from the F0a litters that were outside the mean weaning dates for observation of sexual maturation only (F1-select). All F1 generation animals were dosed for a minimum of 12 weeks at doses of 0, 50, 250 or 1000 mg/kg/day in a corn oil suspension. Following this dosing period, the main study F1-main animals were paired within dosing groups and subsequently dosed throughout mating, gestation and lactation to yield the F2 litters. At weaning of the F2 generation, all surviving F1 adults and F2 pups were sacrificed.

EC No. 415-930-6 contains approximately 3.8 wt% residual tetrapropenyl phenol. The converted approximate equivalent residual concentration TPP present in each test material dose of 0, 50, 250 and 1000 mg/kg/day is (0), (1.9), (9.5) and (38) mg/kg/day respectively. Where presented, TPP dose appears in parenthesis to distinguish TPP dose from test article dose.

Summary of No Observed Adverse Effect Level

End Point/Generation	NOAEL (mg/kg bw/day)
Parental (F0, F1)	50 (1.9 mg TPP/kg bw/day)
Reproduction	250 (9.5 mg TPP/ kg bw/day)
Offspring (F1, F2)	50 (1.9 mg TPP/kg bw/day)

Results

Clinical Observations

- No deaths were attributed to treatment in this study
- Salivation was noted predominantly post-dosing at all dose levels in F0 and F1 animals of both sexes. This effect was considered a response to oral dosing of a material with an unpleasant taste.

Body Weight

- F0 and F1 males treated at 1000 mg/kg/day (38 mg/kg/day TPP)
    - F0 males
        - Significantly decreased absolute body weight was evident beginning at study week 12 (546 ± 49.4 g vs. control of 588 ± 59.0 g) and persisted through termination at week 25 (634 ± 60.0 vs. control 702 ± 75.0 g).
        - Overall body weight gain was 90% of that seen for control males.
    - F1 males
        - Decreased absolute body weight was evident after about 6 weeks of dosing and throughout the remainder of the study but never achieved statistical significance (568 ± 42.2 vs. 606 ± 69.6g).
        - Overall body weight gain was 93% of that seen for control males.
- F0 and F1 females - No significant effect on body weight or body weight gain was observed in any treatment group during any stage of the study.

Food Consumption

- Food consumption was sporadically increased compared to control in F0 males treated at 1000 mg/kg/day (38 mg/kg/day TPP) and 250 mg/kg/day (9.5 mg/kg/day TPP).
- Group mean food consumption were higher compared to control in F1 males treated at 1000 mg/kg/day (38 mg/kg/day TPP) during the latter weeks of dosing prior to pairing (Weeks 26, 27, and 29)

  Note: The study director did not consider these findings toxicologically significant.
- F0 and F1 females treated at 1000 mg/kg/day (38 mg/kg/day TPP)
    - Food consumption was increased during maturation compared to controls. This effect emerged in the later stages of this study phase.
    - Food consumption was increased during gestation compared to controls. This effect emerged in the later stages of this study phase (weeks 2 and 3).
    - Reduced food consumption was noted in F1 females compared to controls during lactation.

      Note: The study director attributed this difference during lactation to the smaller litter sizes of this dose group.
    - No differences in food consumption were noted at lower doses in either generation.

Hematology

- F0 males treated at
    - 1000 mg/kg/day (38 mg/kg/day TPP):
        - Increased Activated Partial Thromboplastin Time (18.1 ± 4.46 vs. 15.0 ± 2.50 secs)
        - Reduced red blood cell count – (7.50 ± 1.79 vs. 8.42 ± 0.33 1012/l)

NOTE: this finding was not statistically significant

- 250 mg/kg/day (9.5 mg/kg/day TPP)
  - Increased Activated Partial Thromboplastin Time - (17.5 ± 3.63 vs. 15.0 ± 2.50 secs)
  - Reduced red blood cell count - (8.07 ± 0.49 vs. 8.42 ± 0.33 1012/l)
- 50 mg/kg/day (1.9 mg/kg/day TPP):
  - No significant treatment-related differences were observed

- F0 females treated at
  - 1000 mg/kg/day (38 mg/kg/day TPP):
    - Increased clotting time (CT) – (22.8 ± 1.60 vs. 21.3 ± 1.98 sec)
  - 250 mg/kg/day (9.5 mg/kg/day TPP):
    - No significant treatment-related differences were observed
  - 50 mg/kg/day (1.9 mg/kg/day TPP):
    - No significant treatment-related differences were observed

<u>Serum Chemistry</u>

- F0 males treated at
  - 1000 mg/kg/day (38 mg/kg/day TPP)
    - Increased alkyline phosphatase (AP) (503 ± 215.9 vs. 388 ± 100 IU/l)
  - 250 mg/kg/day (9.5 mg/kg/day TPP)
    - Decreased Creatinine - 0.60 ± 0.057 vs. 0.64 ± 0.053 secs, treated vs. control, respectively
  - 50 mg/kg/day (1.9 mg/kg/day TPP):
    - No significant treatment-related differences were observed

- F0 females treated at
  - 1000 mg/kg/day (38 mg/kg/day TPP)
    - Increased alanine aminotransferase (ALAT) – 71 ± 37.6 vs. 47 ± 12.7 IU/l, treated vs. control, respectively*
    - Increased blood urea – 39 ± 8.0 vs. 32 ± 52 mg/dl, treated vs. control, respectively
    - Increased phosphorus (P) – 2.31 ± 0.628 vs. 1.73 ± 0.553 mmol/l, treated vs. control, respectively
    - Increased calcium (Ca++) – 3.01 ± 0.298 vs. 2.81 ± 0.260 mmol/l, treated vs. control, respectively
  - 250 mg/kg/day (9.5 mg/kg/day TPP):

- No significant treatment-related differences were observed

- 50 mg/kg/day (1.9 mg/kg/day TPP):
  - No significant treatment-related differences were observed

  **NOTE: This finding is high due to very high values in a limited number of treated animals, which are probably statistical outliers rather than findings. Note the very large standard deviation of the treated group.*

Organ Weights

- F0 males treated at
  - 1000 mg/kg/day (38 mg/kg/day TPP):
    - Increased group mean brain weight relative to body weight (0.3398 ± 0.03247 vs. 0.3082 ± 0.03519)
    - Increased group mean liver weight relative to body weight (4.1087 ± 0.31869 vs. 3.5240 ± 0.28747%)
    - Increased group mean kidney weight, absolute and relative to body weight (5.026 ± 0.7830 vs. 4.513 ± 0.3887 g and 0.7861 ± 0.06785 vs. 0.6438± 0.06476%, respectively)
    - Increased group mean spleen weight relative to body weight (0.1413 ± 0.01931 vs. 0.1251 ± 0.01165)
    - Decreased group mean left epididymis weight relative to body weight (0.1159 ± 0.02085 vs. 0.1060± 0.01389%)
    - Decreased group mean right epididymis weight relative to body weight (0.1257 ± 0.01684 vs. 0.1107 ± 0.01697 %)
    - Increased group mean left testis weight relative to body weight (0.3090 ± 0.04493 vs. 0.2932± 0.04004%)
    - Increased group mean right testis weight relative to body weight (0.3199 ± 0.03532 vs. 0.2930 ± 0.04111 %)
  - 250 mg/kg/day (9.5 mg/kg/day TPP):
    - Increased group mean liver weight, absolute and relative to body weight (28.140 ± 4.0914 vs. 24.893 ± 3.4940 g and 3.8087 ± 0.32993 vs. 3.5240 ± 0.28747%)
    - Increased group mean absolute kidney weight (4.970 ± 0.482 vs. 4.513 ± 0.3887 g)
  - 50 mg/kg/day (1.9 mg/kg/day TPP):
    - No significant treatment-related differences in organ weight, absolute or relative, were observed.
- F1 males (main study) treated at
  - 1000 mg/kg/day (38 mg/kg/day TPP):

        - Increased group mean brain weight relative to body weight (0.3783 ± 0.02793 vs. 0.33499 ± 0.03422)
        - Increased group mean adrenal gland weight relative to body weight (0.0124 ± 0.0022 vs. 0.0102 ± 0.00254)
        - Increased group mean liver weight relative to body weight (4.6482 ± 0.4132 vs. 4.0844 ± 0.3112 %)
        - Increased group mean kidney weight, absolute and relative to body weight (4.942 ± 0.4629 vs. 4.473 ± 0.5417 g and 0.8573 ± 0.07373 vs. 0.7306 ± 0.07199 %)
        - Increased group mean left testis weight relative to body weight (0.3372 ± 0.04789 vs. 0.3083± 0.03839%)
        - Decreased group mean right testis weight relative to body weight (0.3314 ± 0.04789 vs. 0.3020 ± 0.05218 %)
    - 250 mg/kg/day (9.5 mg/kg/day TPP):
        - No significant treatment-related differences in organ weight, absolute or relative, were observed
    - 50 mg/kg/day (1.9 mg/kg/day TPP):
        - No significant treatment-related differences in organ weight, absolute or relative, were observed

- F0 females treated at
    - 1000 mg/kg/day (38 mg/kg/day TPP):
        - Increased group mean liver weight, absolute and relative to body weight (21.281 ± 1.8005 vs. 19.558 ± 1.6811 g and 5.3733 ± 0.46695 vs. 4.7675 ± 0.38786 %)
        - Increased group mean adrenal gland weight relative to body weight (0.0242 ± 0.00317 vs. 0.0214 ± 0.00302 %)
    - 250 mg/kg/day (9.5 mg/kg/day TPP):
        - No significant treatment-related differences in organ weight, absolute or relative, were observed
    - 50 mg/kg/day (1.9 mg/kg/day TPP):
        - No significant treatment-related differences in organ weight, absolute or relative, were observed

- F1 females (main study) treated at
    - 1000 mg/kg/day (38 mg/kg/day TPP):
        - Increased group mean liver weight, absolute and relative to body weight (20.616 ± 2.1548 vs. 18.876 ± 1.7455 g and 5.5012 ± 0.44061 vs. 5.1885 ± 0.36711 %)
        - Increased group mean ovaries weight relative to body weight (0.0301 ± 0.00381 vs. 0.0349 ± 0.00746 %)

- 250 mg/kg/day (9.5 mg/kg/day TPP):
  - No significant treatment-related differences in organ weight, absolute or relative, were observed
- 50 mg/kg/day (1.9 mg/kg/day TPP):
  - No significant treatment-related differences in organ weight, absolute or relative, were observed

Histological Changes

- F0 generation - no microscopic changes were considered treatment related in any dose group
  - Three of the males from the 250 mg/kg/day dose group (9.5 mg/kg/day TPP) that failed to produce litters were found to have testicular epithelial degeneration or an absence of germinal epithelium. This was not attributed to the test substance as it did not occur in a dose-responsive manner and was not observed in the F1 males at this dose level.
- F1 generation - no microscopic changes were considered treatment related in any dose group
  - At 1000 mg/kg/day, a low incidence of aspermia in males and a low incidence of females with no ovarian corpora lutea were noted.
- F2 generation - no microscopic changes were considered treatment related in any dose group

Other general macropathology findings:

No other significant or treatment-related macroscopic findings were noted in the study overall.

Reproductive performance and fertility

- F0 generation
  - Dosed at 1000 mg/kg/day (38 mg/kg/day TPP)
    - Pregnancy index was reduced in the F0a mating phase. (77.8% vs. 100% controls)
    - Pregnancy index was reduced in the F0b mating phase. (80.8% vs. 96.4% controls)
      - One male failed to produce a litter in either mating phase
      - Two females failed to produce a litter in either mating phase
    - No effect on pre-coital interval as noted in either mating phase
  - Dosed at 250 mg/kg/day (9.5 mg/kg/day TPP)
    - Pregnancy index was reduced in the F0a mating phase. (71.4% vs. 100% controls)
    - Pregnancy index was reduced in the F0b mating phase. (75.0% vs. 96.4% controls)
      - Four males failed to produce a litter in either mating phase
      - Two females failed to produce a litter in either mating phase
    - No effect on pre-coital interval as noted in either mating phase

Note: The study director did not consider the reduced fertility observed at the 50 or 250 mg/kg/day levels to be due to test substance administration, as the findings were not repeated in the second generation reproduction. The effect at the highest dose, 1000 mg/kg/day, repeated in the second generation and was attributed to the test substance exposure.

- Dosed at 50 mg/kg/day (1.9 mg/kg/day TPP)
  - Rate of non-pregnancies was considered within normal limits for the F0a mating phase (92.8% vs. 100% controls)
  - Pregnancy index for F0b mating phase was 82.1% vs. 96.4% controls.
    - Two males failed to produce a litter in either mating phase
    - Zero females failed to produce a litter in either mating phase
  - No effect on pre-coital interval as noted in either mating phase

- F1 generation
  - Dosed at 1000 mg/kg/day (38 mg/kg/day TPP)
    - Pregnancy rate was reduced compared to controls (84.0% vs. 96.4% controls)
    - One mating pair failed to show evidence of mating.
    - No effect on pre-coital interval as noted
    - No effects were observed in the other dose groups

<u>Gestation</u>

- F0 generation dosed at 1000 mg/kg/day (38 mg/kg/day TPP)
  - One female sacrificed in extremis with finding consistent with dystocia
- F1 generation dosed at 1000 mg/kg/day (38 mg/kg/day TPP)
  - Three females found dead with findings consistent with dystocia
  - One female sacrificed in extremis with findings consistent with dystocia

Note: No instances of dystocia were identified at any other treatment level in either generation. The study director did not consider the observations in the high dose groups to be treatment-related because no effect on gestation length was observed.

<u>Sexual Maturation</u>

- F1 generation males (sexual maturation study group)
  - Dosed at 1000 mg/kg/day (38 mg/kg/day TPP)
    - Significant increase in male body weight at time of sexual maturation (220 ± 14.5 vs. 201 ± 17.1 g, treated compared to control, respectively)

        - Slight, but not statistically significant, increase in age at time of sexual maturation (44 ± 1.5 vs. 43 ± 1.6 days, treated compared to control, respectively)
    - Dosed at 250 mg/kg/day (9.5 mg/kg/day TPP)
        - Significant increase in male body weight at time of sexual maturation (216 ± 19.7 vs. 201 ± 17.1 g, treated compared to control, respectively)
        - Significant increase in age at time of sexual maturation (45 ± 1.3 vs. 43 ± 1.6 days, treated compared to control, respectively)
    - Dosed at 50 mg/kg/day (1.9 mg/kg/day TPP)
        - Slight, not statistically significant, increase in male body weight at time of sexual maturation (215 ± 29.6 vs. 201 ± 17.1 g, treated compared to control, respectively)
        - Slight, but not statistically significant, increase in age at time of sexual maturation (44 ± 2 vs. 43 ± 1.6 days, treated compared to control, respectively)
- F1 generation females (sexual maturation study group) - no difference in age or body weight at sexual maturation was noted at any dose level

  Note: Delayed sexual maturation with increased body weight at maturation in male rats is not a finding that has been attributed to TPP exposure in studies with dosages up to 75 mg/kg/day, while accelerated sexual maturation in females has been observed at such exposures. The delay in male sexual maturation observed in this study is attributed to the test substance, EC 415-930-6, but is not attributed to the TPP content in the substance as it conflicts with TPP findings.

<u>Female Reproductive Toxicity</u>

- F0 generation
    - Dosed at 1000 mg/kg/day (38 mg/kg/day TPP)
        - No significant effects on estrous cycle compared to controls
        - No difference in total corpora lutea compared to control
        - No difference in number of implantation sites compared to control
        - No difference in pre- or post-implantation loss compared to control (NOTE that post-implantation loss was comparatively high in the controls)
        - Slight, but not statistically significant, group mean live litter size was reduced compared to control (12.0 ± 2.4 vs. 13.5 ± 3.9 born)
    - 250 and 50 mg/kg/day dose levels (9.5 and 1.9 mg/kg/day TPP, respectively)
        - No effects noted on estrous cycle compared to controls
        - No difference in total corpora lutea compared to control
        - No difference in number of implantation sites compared to control
        - No difference in pre- or post-implantation loss compared to control
- F1 generation (main study)

- Dosed at 1000 mg/kg/day (38 mg/kg/day TPP)
  - No significant effects on estrous cycle compared to controls
  - Group mean live litter size was reduced compared to control (12.3 ± 2.9 vs. 14.4 ± 2.4 born)
  - Group mean live litter size was reduced compared to control on Day 1 post-partum (12.2 ± 3.0 vs. 14.2 ± 2.6 born)
- Dosed 250 and 50 mg/kg/day dose levels (9.5 and 1.9 mg/kg/day TPP, respectively)
  - No effects noted on estrous cycle compared to controls
  - No difference in total corpora lutea compared to control
  - No difference in live litter size

Male Reproductive Toxicity

- F0 generation - all dose levels
  - No significant effects on concentration, motility or morphology of sperm
  - No difference in mean homogenization-resistant spermatic count at any dose in testes or cauda epididymis

Discussion and Conclusions

EC No. 415-930-6 dosed at 1000 mg/kg/day (38 mg/kg/day TPP) was associated with reduction in body weight and body weight gain in males, but not females of the F0 and F1 generations. Food consumption was increased in females of both generations in later weeks of maturation and gestation, but not throughout the duration of the study. The Study Director speculated that these observations could reflect an impact on food efficiency.

An increase in non-pregnancies was observed in the F0a mating phase at 1000 and 250 mg/kg/day (38 and 9.5 mg/kg/day TPPs, respectively). A subsequent re-mating of alternative mating pairs in the F0b phase replicated these findings and resulted in an increase in non-pregnancies at 50 mg/kg/day (1.9 mg/kg/day TPP). Analysis of individual mating performance for all animals indicated that few animals failed to produce at least one litter and there was no evidence of an effect on fertility isolated to one sex. An increase in non-pregnancies was also seen in F1 pairs treated at 1000 mg/kg/day; however, there was no replication of effects at 250 or 50 mg/kg/day. No histological findings in reproductive tissues were noted in males or females of either generation. No effect on semen parameters was noted in either generation. These effects did not follow a dose responsive pattern in this study. Due to the absence of fertility effects in the second generation at the low and middle dosages, while the effect at the highest dose recurred, the study director did not interpret fertility effects at any dosage except 1000 mg/kg/day.

A slight reduction in live litter size at birth was observed in F0 and F1 animals treated at 1000 mg/kg/day, but no difference in pup viability was observed at any dose in either generation.

An increase in age at sexual maturation and increased body weight at sexual maturation were noted in male offspring of the F0 generation dosed at 1000 and 250 mg/kg/day (38 and 9.5 mg/kg/day TPP, respectively). This parameter was not measured in the F2 offspring. No effect on age or weight at sexual maturation was seen in females of either generation.

While a limited number of additional statistically significant tissue effects were observed in discrete subsets of the tested population, no organ weight changes were correlated with histological findings in any instance.

Two Generation Oral (Gavage) Reproductive Toxicity Study in the Rat (Wood *et al.*, 2003)

Study Design

This study, originally designed to meet or exceed the OECD 415 Guideline (1983), evaluated the effects of the test material (EC No. 430-180-1) on growth and reproductive performance in rat (Sprague-Dawley Crl:DC IGS BR). A second generation was added to the study design as a result of findings in the first generation offspring, thus modifying the protocol such that the final study meets or exceeds the OECD 416 Guideline.

Test material was administered orally by gavage to the F0 generation (28 males and 28 females per group) at doses of 0, 5, 30 or 150 mg/kg/day in a corn oil suspension (dose volume of 5 ml/kg) for at least 10 weeks prior to mating and then to dose-matched mating pairs throughout the mating, gestation and lactation phases. At weaning, F1 offspring from each dose group were selected to proceed into the main study. A separate collection of pups were selected from the F0 litters that were outside the mean weaning dates for observation of sexual maturation only. All F1 generation animals were dosed for a minimum of 10 weeks at doses of 0, 5, 30 or 150 mg/kg/day in a corn oil suspension. Following this dosing period, the main study F1 animals were paired within dosing groups and subsequently dosed throughout mating, gestation and lactation to yield the F2 litters. At weaning of the F2 generation, all surviving F1 adults and F2 pups were sacrificed.

EC No. 430-180-1 contains approximately 26 wt% residual tetrapropenyl phenol. The converted approximate equivalent residual concentration of TPP present in each test material dose of 0, 5, 30 and 150 mg/kg/day are 0, 1.3, 7.8 and 39 mg/kg/day, respectively.

Summary of No Observed (Adverse) Effect Levels

End Point/Generation	NO(A)EL (mg/kg bw/day)
Fertility Performance (F0 & F1)	150 – NOAEL (39 mg TPP/kg bw/day
Reproductive Performance (F0 & F1)	150 – NOAEL (39 mg TPP/kg bw/day)
Offspring Effects (viability and growth)	30 – NOEL (7.8 mg TPP/kg bw/day)
Parental	<5 (<1.3 mg TPP/kg bw/day)

Results

Clinical Observations

- There were no deaths attributed to treatment.
- Salivation was noted predominantly post-dosing at 150 mg/kg/day in F0 and F1 animals of both sexes. This effect was considered a response to oral dosing of a material with an unpleasant taste.

Body Weight

- A slight reduction in body weight gain was observed in F0 and F1 males treated at 150 mg/kg/day (39 mg/kg/day TPP) but not at any other treatment level.
- No significant effect was observed in females of any generation or any treatment group.

Food Consumption

- There were no significant treatment-related differences in male or female food consumption for either F0 or F1 generation during maturation or in males post-maturation.
- There were no significant treatment-related effects on female food consumption compared to controls for either generation during gestation.
- There were no significant treatment-related effects on female food consumption compared to controls for either generation during lactation.

  NOTE: The study director notes that while there were occasional significant differences between a treated group and controls, the findings were not indicative of a treatment-related trend.

Hematology:

Not evaluated as part of this study.

Serum Chemistry

Not evaluated as part of this study

Organ Weights

- F0 males treated at
  - 150 mg/kg/day (39mg/kg/day TPP):
    - Increased liver weight relative to body weight (3.807 ± 0.2714 vs. 3.553 ± 0.2818%)
    - Increased kidney weight relative to body weight (0.720 ± 0.0.0593 vs. 0.675± 0.0488%
    - Decreased group mean seminal vesicle/ coagulating gland weight (absolute and relative to body weight, 2.335 ± 0.3813 vs. 2.726 ± 0.3839 g and 0.384 ± 0.0830 and 0.435 ± 0.062%, respectively)
    - Decreased group mean left epididymis absolute weight (0.683 ± 0.0655 vs. 0.738 ± 0.0732 g)
  - 30 mg/kg/day (7.8 mg/kg/day TPP):
    - Decreased group mean seminal vesicle/ coagulating gland weight (absolute and relative to body weight, 2.480 ± 0.3451 vs. 2.726 ± 0.3839 g and 0.402 ± 0.0559 and 0.435 ± 0.062%, respectively)
    - Decreased group mean left epididymis absolute weight - (0.687 ± 0.0768 vs. 0.738 ± 0.0732 g)

- 5 mg/kg/day (1.3 mg/kg/day TPP):
  - Decreased mean seminal vesicle/ coagulating gland weight relative to body weight (0.394 ± 0.0619 and 0.435 ± 0.062%)

- F1 males treated at:
  - 150 mg/kg/day (39mg/kg/day TPP):
    - Increased group mean liver weight relative to body weight (4.048 ± 0.3851 vs. 3.585 ± 0.3007 %)
    - Increased group mean kidney weight relative to body weight (0.738 ± 0.0876 vs. 0.643 ± 0.0657 %)
    - Decreased group mean seminal vesicle/ coagulating gland weight (absolute and relative to body weight, 2.261 ± 0.3972 vs. 2.761 ± 0.4991g and 0.392 ± 0.0607 vs. 0.447 ± 0.0698%)
    - Increased group mean thyroid gland weight relative to body weight (0.003 ±0.0011 vs. 0.002 ± 0.008 %)
    - Increased mean pituitary gland weight relative to body weight (0.003 ± 0.0008 vs. 0.002 ± 0.0008)
  - 30 mg/kg/day (7.8 mg/kg/day TPP):
    - Decreased group mean seminal vesicle/ coagulating gland weight (absolute and relative to body weight, 2.371 ± 0.0.3126 vs. 2.761 ± 0.4991g and 0.398 ± 0.0547 vs. 0.447 ± 0.0698%)
    - Statistically significant increased group mean thyroid gland weight, absolute and relative to body weight (0.021 ± 0.0062 vs. 0.016 ± 0.0051 g and 0.004 ± 0.0048 vs. 0.002 ± 0.008%, respectively)
  - 5 mg/kg/day (1.3 mg/kg/day TPP):
    - Decreased mean seminal vesicle/ coagulating gland absolute weight (2.434 ± 0.0.3783 vs. 2.761 ± 0.4991g)
    - Statistically significant increased group mean thyroid gland weight, absolute and relative to body weight (0.020 ± 0.0061 vs. 0.016 ± 0.0051 g and 0.004 ± 0.001 vs. 0.002 ± 0.008%, respectively)

- F0 females treated at
  - 150 mg/kg/day (39 mg/kg/day TPP):
    - No significant treatment-related differences in organ weight, absolute or relative, were observed
  - 30 mg/kg/day (7.8 mg/kg/day TPP):
    - Increased mean thyroid weight relative to body weight (0.005 ± 0.0015 vs. 0.004 ± 0.0014%)

- 5 mg/kg/day (1.3 mg/kg/day TPP):
  - Increased mean thyroid gland weight relative to body weight (0.005 ± 0.0018 vs. 0.004 ± 0.0014%)

- F1 females treated at
  - 150 mg/kg/day (39 mg/kg/day TPP):
    - Increased group mean uterine/uterine cervix weight, absolute and relative to body weight (0.546 ± 0.136 vs. 0.424 ± 0.10 g and 0.153 ± 0.033 vs. 0.117 ± 0.028%, respectively)

      Note: As this result was not correlated to alternations in estrus cycle and was not observed in the F0 generation, the study director considered it to be a chance result.
    - Slight differences in the proportion of growing and antral follicles for one or both ovaries
    - Medium follicles, 0.31 ± 0.36 vs. 1.0 ± 0.51, treated vs. control
    - Large follicles, 0.5 ± 0.46 vs. 1.0 ± 0.46, treated vs. control

      Note: The study director notes that while the differences were statistically significant, the difference represented less than one follicle and therefore is regarded as within the range of acceptable biological variability.
  - 30 mg/kg/day (7.8 mg/kg/day TPP):
    - Decreased mean liver weight, absolute and relative to body weight (16.82 ± 1.82 vs 18.45 ± 1.99 g and 4.77 ± 0.33 vs 5.09 ± 0.38, respectively)
  - 5 mg/kg/day (1.3 mg/kg/day TPP):
    - No significant treatment-related differences in organ weight, absolute or relative, were observed

<u>Histological Changes</u>

- F0 generation - no histological changes were considered treatment related in any dose group
- F1 generation - no histological changes were considered treatment related in any dose group
- F2 generation - no histological changes were considered treatment related in any dose group

<u>Other general macropathology findings:</u>

- F0 - F1 generation male offspring of the 150 mg/kg/day treatment group had lower mean brain weight compared to controls (1.321 ± 0.1326 vs. 1.407 ± 0.0891 g).

  Note: The study director considered this finding to be statistically fortuitous and not biologically significant.

- Also noted in the F1 generation was an increased homogenization resistant spermatid count in testis (77.5 ± 12.29 vs. 71.2 ± 10.51 x 106/g).

  Note: The study director did consider this finding to be biologically significant.

Female Reproductive Toxicity

- F0 generation dosed at 150 mg/kg/day (39 mg/kg/day TPP)
    - No significant effects on estrous cycle compared to controls
    - No effects on reproductive performance
    - No effects on fertility
    - Slight, but not statistically significant, decrease in mean live litter size (13.0 ± 2.9 vs. 14.4 ± 3.1, treated vs. control)
    - Slight, but not statistically significant, decrease in mean uterine implantation sites (13.7 ± 2.7 vs. 15.2 ± 3.9, treated vs. control)

      Note: The study director noted that the mean live litter size and implantations sites, while slightly reduced, were not statistically significant and fell within the range of normal control values.

- F1 generation (maturation study) dosed at 150 mg/kg/day (39 mg/kg/day TPP)
    - Reduction in age and body weight at time of sexual maturation (31.6 ± 1.8 vs. 34.2 ± 2.9 days, treated compared to control, respectively)
    - Reduction in body weight at time of sexual maturation (92 ± 11.7 vs. 107 ±11.2 g, treated compared to control, respectively)

Male Reproductive Toxicity

- F0 generation dosed at 150 mg/kg/day (39 mg/kg/day TPP)
    - No significant effects on concentration, motility or morphology of sperm
    - No effects on reproductive performance
    - No effects on fertility
- F1 generation (maturation study) dosed at 150 mg/kg/day (39 mg/kg/day TPP)
    - Slight not statistically significant increase in age to completion of sexual maturation (44.7 ± 1.9 vs. 43.8 ± 2.0, treated compared to control, respectively)
    - Slight not statistically significant increase in body weight at completion of sexual maturation (214 ± 25.4 vs. 200 ± 18.2, treated compared to control, respectively)

Note: The study director indicated the relevance of these findings is questionable considering the intergroup variability in age and body weight at sexual maturation.

Discussion and Conclusions

EC No. 430-180-1 dosed at 150 mg/kg/day (39 mg/kg/day TPP) was associated with slight reduction in body weight gain in males but not females of the F0 and F1 generations. Accelerated sexual maturation (reduced time to completion of sexual maturation) and lower body weight at sexual maturation was noted in females of the F1 generation. Males of F0 and F1 generations had reduced seminal vesicle/coagulating gland tissue weights compared to control; although the study director attributed the differences to test substance exposure, there were no microscopic alterations

and no adverse effects associated with these differences. Consequently, the organ weight changes did not affect the NOAEL for reproduction and fertility. Also in the F1 generation, increased homogenization resistant spermatid count in testis was observed. While a limited number of additional statistically significant tissue effects were observed in discrete subsets of the tested population, no organ weight changes were correlated with histological findings in any instance.

One-Generation Gavage Reproduction Study (Knapp *et al.*, 2008)

Study Design

A one-generation study, designed to meet or exceed testing requirements of the OECD 415 Guideline, investigated potential adverse effects on reproduction of {EC No. 455-880-2} administered by gavage to Crl:CD(SD) rats (30/sex/group). Both sexes of the parental generation were treated with test material at concentrations of 0 (corn oil vehicle), 50, 170 and 500 mg/kg/day for 70 consecutive days prior to mating. Males continued to receive test article through the period of mating until sacrifice for a total exposure period of 133 to 139 days. Females continued to receive test article through mating, and then through gestation and lactation day 21, up to sacrifice for a total exposure period of 128 to 133 days. The F1 generation was necropsied on postnatal day (PND) 21.

EC No. 455-880-2 contains approximately 2.5 wt% residual tetrapropenyl phenol. The converted approximate equivalent residual concentration TPP present in test material doses of 0, 50, 170 and 500 mg/kg/day are 0, 1.25, 4.25 and 12.5 mg/kg/day, respectively.

Based on the absence of adverse effects on male and female reproductive endpoints, the no-observed-adverse-effect level (NOAEL) for F0 reproductive toxicity for this study was identified as 500 mg/kg/day (12.5 mg/kg/day TPP), the highest dose tested. Based on changes in hematological parameters (prothrombin clotting time), the NOAEL for F0 systemic toxicity was identified as 170 mg/kg/day (4.25 mg/kg/day TPP). Due to the absence of neonatal toxicity at all dose levels, the NOAEL for F1 neonatal developmental toxicity was identified as the highest dosage tested, 500 mg/kg/day (12.5 mg/kg/day TPP).

Summary of No Observed Adverse Effect Levels

End Point/Generation	NOAEL (mg/kg bw/day)
Reproductive toxicity (F0)	500 (12.5 mg TPP/kg bw/day)
Systemic Toxicity (F0)	170 (4.25 mg TPP/kg bw/day)
Developmental Toxicity (F1)	500 (12.5 mg TPP/kg bw/day)

Results

Clinical Observations

- There were no treatment related deaths.
- Frequent instances of salivation and red discoloration around the mouth was noted one hour post-dosing in F0 males and females treated at 170 and 500 mg/kg/day (4.25 and 12.5

mg/kg/day TPP). While both effects were attributed to the test article, only the red discoloration was considered adverse and insufficient for determination of the NOAEL.

Body Weight and Food Consumption

- There were no test article related effects on mean male body weights or body weight gains in the 50, 170 or 500 mg/kg/day groups.
- Statistically significant ($p<0.05$) difference from the control group was an increase in mean body weight gain in the 50 mg/kg/day group during study days 35-42; no dose-response relationship was evident.
- Spurious occurrences of altered food consumption and food efficiency were recorded, but were not considered toxicologically significant in the study.
- Transient differences in food consumption from control were attributed to the test material but were not considered adverse by the study director (no decline in mean body weight was observed).

Hematology:

- 500 mg/kg/day (12.5 mg/kg/day TPP)
  - F0 males treated vs. control values with % change from control
    - Increased Prothrombin Time (20.7 ± 4.04 vs. 15.5 ± 2.11 secs, +33.5% Δ)
    - Increased Activated Partial Thromboplastin Time (23.4 ± 2.4 vs. 18.9 ± 1.88 secs, +23.8 % Δ)
    - Reduced red blood cell count (8.78 ± 0.34 vs. 9.09 ± 0.409 x 106/µl, -3.4% Δ)
    - Decreased, but not statistically significant, absolute reticulocyte count (142.7 ± 26.17 vs. 153.5 ± 22.77 thousand/ µl, -7.0% Δ)
  - F0 females – no significant differences in hematologic parameters was noted

  Note: The study director attributed the changes in prothrombin time but not cell counts to the test article in this dose group.
- 170 mg/kg/day (9.5 mg/kg/day TPP)
  - F0 males treated vs. control values with % change from control
    - Increased, but not statistically significant, mean Prothrombin Time (16.9 ± 2.53 vs. 15.5 ± 2.11 secs, +9% Δ)
    - Increased mean Activated Partial Thromboplastin Time (20.8 ± 2.46 vs. 18.9 ± 1.88 secs, +10.1 % Δ)
    - Decreased mean absolute reticulocyte count (138.9 ± 20.64 vs. 153.5 ± 22.77, -9.5% Δ), which was not attributed to test material
  - F0 females – no significant differences in hematologic parameters was noted

Note: The study director did not consider changes in the 170 mg/kg/day group biologically or toxicologically relevant because most changes in this dose group were within 10% of control.

Serum Chemistry

- 500 mg/kg/day (12.5 mg/kg/day TPP)
  - F0 males treated vs. control with % change from control
    - Increased mean alkaline phosphatase (152. ± 51.9 vs. 99. ± 24.7 U/l, +53.5% Δ)
  - F0 females treated vs. control with % change from control
    - Increased mean alkaline phosphatase (96. ± 34.0 vs. 75. ±19.3 U/l, +28.0% Δ)
    - Decreased mean cholesterol – (66. ± 9.9 vs. 79. ± 14.6 mg/dl, -16.5% Δ)

- 170 mg/kg/day (9.5 mg/kg/day TPP)
  - F0 males –
    - Decreased mean aspartate aminotransferase (80. ± 12.7 vs. 99. ± 39.9 U/l, -19.2% Δ)
  - F0 females – no significant differences in hematologic parameters noted

Note: The study director did not consider the changes in serum chemistry adverse due to the absence of microscopic correlations. The study director did not consider the change in aspartate aminotransferase test article related because there was no dose-related trend to this change.

Organ Weights

- F0 males treated at 500 mg/kg/day (12.5 mg/kg/day equivalent):
  - Absolute mean organ weight, treated vs. control with % change
    - Increased mean absolute liver weight (19.32 ± 2.706 vs. 17.14 ± 2.089, +12.7% Δ)
    - Increased mean absolute kidney weight (4.47 ± 0.555 vs. 4.00 ± 0.441, +11.8% Δ)
    - Increased mean absolute adrenal gland weight (0.0637 ± 0.00988 vs. 0.0563 ± 0.0108, +13.1% Δ)
    - Increased mean absolute pituitary gland weight (0.0.0157 ± 0.00380 vs. 0.0129 ± 0.0184, +21.7% Δ)
  - Mean organ weight relative to terminal body weight, treated vs. control
    - Increased relative mean liver weight (3.495 ± 0.2430 vs. 2.981 ± 0.3343)
    - Increased relative mean kidney weight (0.812 ± 0.0787 vs. 0.695 ± 0.0791)
    - Increased relative mean adrenal glands weight (0.012 ± 0.0019 vs. 0.010 ± 0.0019)
    - Increased relative mean pituitary gland weight (0.003 ± 0.0007 vs. 0.002 ± 0.0004)
  - Mean organ weight relative to brain weight, treated vs. control

        - Increased relative mean liver weight (908.550 ± 113.723 vs. 812.335 ± 105.0909)
        - Increased relative mean kidney weight (210.167± 20.8327 vs. 189.382 ± 22.0756)
        - Increased relative mean adrenal glands weight (3.001 ± 0.4702 vs. 2.664 ± 0.4588)
        - Increased relative mean pituitary gland weight (0.737± 0.1667 vs. 0.610 ± 0.0742)
- F0 males treated at 170 mg/kg/day (4.25 mg/kg/day TPP):
    - Mean organ weight relative to terminal body weight, treated vs. control
        - Increased mean liver weight (3.154 ± 0.2029 vs. 2.981 ± 0.3343)
- F0 females - No statistically significant difference in mean absolute or relative organ weights observed at any dose

Histological Changes

F0 generation - no histological changes were considered treatment related in any dose group

Other general macropathology findings:

No additional macropathology findings were observed in any dose group.

Reproductive performance and fertility

- All dose levels
    - Reproductive performance for both sexes was similar to control for all dose levels
    - Mating indices for both sexes were not significantly different from control at any dose
    - Fertility indices were not significantly different from control at any dose
    - Male copulation indices and female conception indices were not significantly different from controls at any dose
    - Pre-coital period was similar to control for all mating pairs at all dose levels
    - There was no evidence of dystocia in treated females at any dose level

Female Reproductive Toxicity

- All dose levels
    - No effect on estrous cycle length was observed at any dose
    - No effect on gestation length was observed at any dose
    - No significant difference in implantation sites at any dose
    - No significant difference in unaccounted for sites at any dose
    - No difference in the mean number of pups born
    - No differences in mean live litter size on PND 0
    - No difference in number of males born

- No difference in male pup survival post-natal

Note: According to the study director, a slight increase in pup deaths seen in the 50 and 500 mg/kg/day (1.25 and 12.5 mg/kg/day TPP) dose groups showed no dose-related pattern and was not evident when the data were evaluated on a litter proportion basis. The study director concluded the slight increase in pup loss was not related to the test article.

Male Reproductive Toxicity

- All dose levels
  - No significant effects on concentration, motility or morphology of sperm
  - No difference in mean homogenization spermatic count at any dose in testes or epididymides

Discussion and Conclusions

Systemic Effects

In the parental generation a transient decline in mean body weight gain compared to controls was observed on study days 0 – 7 in females treated with 500 mg/kg/day. No other effect on body weight or body weight gain was noted for the duration of the study at any dose for either sex. No reduction in food consumption or food efficiency was seen in any dose group.

In males treated at 500 mg/kg/day (12.5 mg/kg/day TPP) increased mean prothrombin time was noted. An increase in mean Activated Partial Prothrombin Time was also noted in males at the 170 and 500 mg/kg/day dose levels (4.25 and 12.5 mg/kg/day TPP, respectively). However, the increase observed in the 170 mg/kg/day (4.25 mg/kg/day TPP) was sufficiently small that the study director considered it to be biologically or toxicologically irrelevant. Mean alkaline phophatase levels were increased in males and females treated at 500 mg/kg/day (12.5 mg/kg/day TPP) and serum cholesterol was reduced in females of this dose group. No microscopic correlation to these findings were noted. A reduced aspartate aminotransferase level was observed in the 170 mg/kg/day group (4.25 mg/kg/day TPP) males but no other dose group of either sex. This finding was not considered test article-related.

Mean absolute and relative (to brain) liver and kidney weights were increased in males dosed at 500 mg/kg/day (12.5 mg/kg/day TPP) while mean absolute and relative (to brain) liver weight was also increased at the 170 mg/kg/day dose (4.25 mg/kg/day TPP) in males. The findings noted here were not correlated to microscopic findings in any instance and therefore not considered adverse by the study director. Increased absolute and relative adrenal and pituitary gland weights were also seen in males dosed at 500 mg/kg/day (12.5 mg/kg/day TPP). These findings were attributed to general physiological stress and not the test article by the Study Director.

Reproductive Performance and Effects on Fertility

Male and female reproductive performance was not affected by test article administration at any dose. Female estrous cycle length and gestation length were not affected by test article administration at all dosage levels and spermatogenic endpoints were also unaffected by test article administration at all doses. No test article-related effects were observed on the mean number of implantation sites or the number of unaccounted-for sites at any dose level.

Administration of the test article to the parental animals did not affect the mean numbers of pups born, live litter size on PND 0, pup sex ratio or postnatal survival in any dose group. The general

physical condition of the F1 pups was not affected by F0 test article administration. No test article-related effects on F1 pup body weights or body weight gains were noted at any dosage level. No macroscopic findings were noted in the F1 pups that were found dead or those examined at the scheduled necropsy attributable to F0 parental treatment with the test article.

Proposed Specific Concentration Limit for TPP

Data from TPP and TPP-derived substances support a SCL of 1.5% for reproductive toxicity.

Toxicological data for TPP were evaluated to identify a concentration limit for TPP unassociated with reproductive effects. The SCL of 1.5% was identified from the two reproductive toxicity studies conducted with TPP (Edwards et al., 2012; Knapp et al., 2006) and represents the maximum amount of TPP equivalent to 15 mg TPP/kg bw/day that has no effects upon reproductive endpoints. In these studies, the most sensitive, consistent alteration to the reproductive system produced by TPP exposure was reduced ovary weight. This change occurred in a dose-responsive manner to exposures as low as 25 mg TPP/kg bw/day in the one-generation reproduction study (Knapp et al., 2006), but did not occur at 15 mg TPP/kg bw/day in either generation of the two-generation study (Edwards et al., 2012). **The SCL value of 1.5% was empirically derived and represents the highest No-Adverse-Effect-Level (NOAEL) for ovary weight derived from TPP reproductive toxicity studies.**

Although these studies also identified effects upon the male reproductive system, reproducible effects to the male system occurred in the presence of systemic toxicity, particularly concurrent with reduction to body weight. A body of evidence (Chapin *et al.,* 1993; Eng *et al.,* 1987; Laws *et al.,* 2007; Rehm *et al.,* 2008) provides a consistent profile that reductions to body weight or growth in the rat impact various organ weights, including organs of the male reproductive system, as well as potential changes to sperm production. The profile of adverse effects due to restricted food consumption was sufficiently similar to the results observed in male rats following TPP exposure that we considered the results as likely to be secondary to systemic health effects in males. Consequently, these endpoints were not modeled.

Utilizing the data of female rats exposed to TPP, two further steps were undertaken in the process of determining a valid, conservative SCL:

1. Determination of ED_{10Low} for ovary weight, and
2. Validation of the proposed SCL to substances that contain TPP.

These steps are described in detail below:

1. Determination of ED_{10Low} for ovary weight. In alignment with ECHA Guidance on the Application of the CLP Criteria (version 3, Nov. 2012), ED analyses were used to perform dose-response evaluation of data generated by exposing rats to TPP in a 90-day dietary study (Haas, 2012), an oral (gavage) one-generation (Knapp, 2006) and a dietary two-generation reproductive toxicity study (Edwards, 2012). The USEPA software BMDS (version 2.3.1) was used to estimate the ED_{10} for ovary weight as a critical endpoint in each study. Only data for which one or more of the dosed groups has a statistically significantly change from control were modeled. While modeling the data on changes in ovary and oviduct weights was attempted, a combination of ovary and oviduct weights was in the 90-day study (Haas 2012) and in the 1-generation study (Knapp

2006). However, in the 2-generation study (Edwards, 2012), the ovary weights were provided separately, with the oviduct weights combined with the weights for the uterus and cervix. Therefore, it was not possible to model the exact same endpoint for all three studies.

The dose-response data that were modeled included:

1. Ovary (with oviduct) Weight

    a. 90 Day Study Females

    b. 1 - Generation Study F0 Females

2. Females Ovary (without oviduct) Weight

    a. 2 - Generation Study F0 Females

    b. 2 - Generation Study F1 Females

All of the continuous models in the BMDS software suite were attempted to be fit to the statistically significant endpoints (Table 30). 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), in the absence of other data suggesting a toxicologically-relevant threshold for changes in ovary weight.

The results of the modeling are summarized in the accompanying table. A stringent adherence to the BMD modeling guidance (USEPA 2000) regarding criteria for model fit was applied (i.e., selection of the appropriate variance model, chi-square p-value criteria for model fits). The model providing the "best fit" to the data, based on Akaike information criteria (AIC), p-value and scaled residual of interest, is shaded.

Table 30: 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_{1std} (mg/kg/day)	LED_{1std} (mg/kg/day)
Haas *et al.*, (2012) (90 Day) Ovary and Oviduct Weight	Exponential2	0.2096	-356.30	1.20	63.1	45.1
	Exponential3	0.2613	-356.15	-0.98	85.6	51.9
	Exponential4	0.2096	-356.30	1.20	63.1	44.3
	Exponential5	0.1243	-354.47	-0.90	87.6	53.7
	Hill	0.1400	-354.65	-0.82	87.6	Failed
	Linear	0.3255	-357.37	1.05	72.3	53.8
	Polynomial	0.1085	-354.26	-1.04	91.7	50.9
	Power	0.2227	-355.83	-1.00	84.9	55.1
Knapp (2006) (1Gen)	Exponential2	0.2744	-763.89	-1.38	64.6	50.1
	Exponential3	0.2744	-763.89	-1.38	64.6	50.1

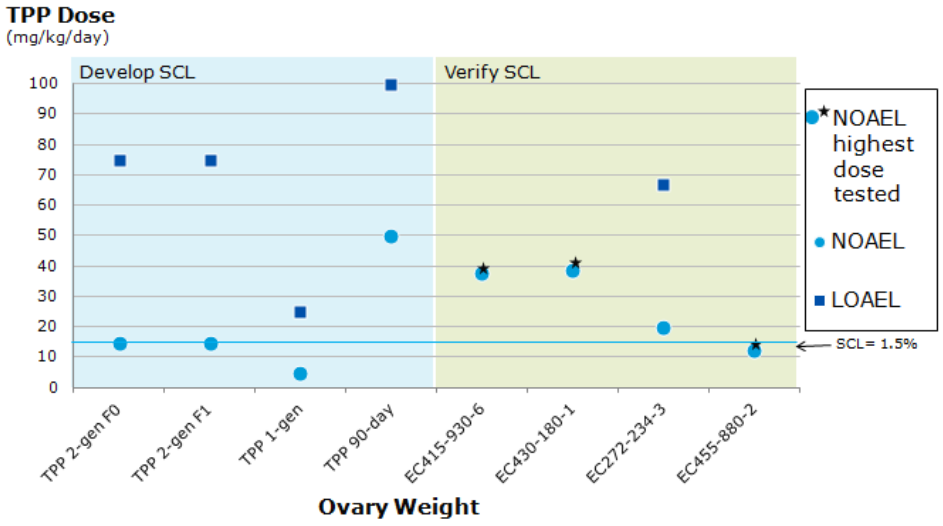
F0 Females Ovary and Oviduct Weight	Exponential4	0.6789	-764.30	-0.11	34.2	18.6
	Exponential5	N/A	-762.48	0.00	32.0	19.0
	Hill	N/A	-762.48	0.00	33.2	18.4
	Linear	0.1894	-763.15	-1.56	71.8	58.0
	Polynomial	0.1894	-763.15	-1.56	71.8	58.0
	Power	0.1894	-763.15	-1.56	71.8	58.0
Edwards *et al.*,(2012) (2 Gen) F0 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 *et al.*, (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 *et al.*, 2012) study (both generations) and the one-generation study (Knapp, 2006), as well as the 90-day study (Haas *et al.*, 2012) result in ED_{10Low} values greater than 1.5%. ED_{10Low} values based upon a parameter of reproductive performance, litter size, resulted in higher values. These ED_{10Low} values were 29 mg/kg/day (2.9%) for the Knapp 2006 study and 67.6 mg/kg/day (6.8%) for the Edwards *et al.*, 2012 study. For the Edwards *et al.*, 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. 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. The potency banding proposed in the ECHA Guidance is considered unnecessary due to the opportunity to validate the SCL to existing test data of substances that contain TPP as an impurity (below).

2. Validation of the proposed SCL for substances that contain TPP. The SCL value of 1.5% was validated against existing test data for TPP-derived test substances that contain 2.5% to 26% TPP. The TPP-derived test substances are UVCB substances that contain TPP as an impurity. The substance identities are fully described in Section 1.2.1.

These substances were tested according to regulatory guideline in effect at the time of each study. TPP-equivalent dosages were 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.

Validation Summary of Proposed Specific Concentration Limit of TPP



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 ED_{10} methodology, is unwarranted for this substance in light of the following considerations:

a. The ED_{10Low} values calculated from the two-generation (Edwards, 2012) reproduction study for TPP were compared to the ED_{10Low} value for the supporting study, and the lowest value was utilized in setting the SCL 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 *et al.*, 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 TPP/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 *et al.*, 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 *et al.*, 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 et al.*, 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/kg/day.

4.11.4 Summary and discussion of reproductive toxicity

In the key study for reproductive toxicity (Edwards *et al.*, 2012), via oral exposure, the following no-observed-adverse-effect levels (NOAELs) have been determined as valid for the two-generation key study.

- Parental NOAEL = 15 and 1.5 mg/kg/day for F0 and F1, respectively.
- Male and female reproductive NOAEL = 15 mg/kg/day
- Neonatal NOAEL = 15 mg/kg/day

In the two-generation reproductive toxicity study (Edwards *et al.*, 2012), findings at 75 mg/kg/day consisted of lower weights in multiple tissues, including the epididymides and cauda epididymides, prostate, seminal vesicles/coagulating glands, and left/right testes in F0 and F1 males treated at 75 mg/kg/day. Mean epididymal sperm concentration was decreased in F0 males at 75 mg/kg/day. Renal mineralization was seen in F0 males at 75 mg/kg/day and F1 males treated at 15 and 75 mg/kg/day, a finding frequently seen in female rats, but less commonly observed in males. Decreased implantation sites (F0 females) and increased estrous cycle lengths (F0 and F1 females) were noted at 75 mg/kg/day. Decreased corpora lutea in F0 and F1 females at 75 mg/kg/day was reported.

In the one-generation reproductive toxicity study (Knapp, 2006), a significant decrease in mean seminal vesicle/ coagulating gland weight relative to brain weight was seen at all dose levels. At 25 mg/kg/day, mean cauda epididymides weight was decreased while testes and epididymides weights were reduced at 125 mg/kg/day. Decreased secretions in the coagulating gland, prostate and seminal

vesicle were observed at 125 mg/kg/day. Additionally, mean epididymides sperm concentration was significantly reduced in the 125 mg/kg/day dose group.

In the 90-day dietary study (Haas *et al.*, 2012), macroscopic findings included small prostates, seminal vesicles, and coagulating glands in 150 and 200 mg/kg/day treatment groups. Additionally, males in the 200 mg/kg/day treatment were also observed to have small testes and epididymides. Absolute and relative prostate and seminal vesicle weights were reduced in all treatment groups above 100 mg/kg/day, while testes weight was increased at 100 and 150 mg/kg/day. Microscopic findings included atrophy of the prostate and coagulating gland in the 200 mg/kg/day dose group. Decreased secretion was noted in the seminal vesicle at the 150 and 200 mg/kg/day dose levels. In this study, findings are observed in multiple categories, including macroscopic changes, microscopic changes, organ weights, and functional measures.

In the 28 day gavage study (Harriman, 2004), macroscopic findings included small testes, prostate, seminal vesicles, and coagulating glands in the 180 and 300 mg/kg/day treatment groups. These observations corresponded to reductions in absolute and relative weights of the testis, prostate, seminal vesicles and epididymides in the same treatment groups. Microscopic findings in these groups included decreased secretion in multiple tissues including prostate, seminal vesicles, and coagulating glands as well as reduced sperm concentration in epididymides. Interstitial cell atrophy was noted in the testes with luminal cell debris present in epididymides, as well. These findings are observed in multiple categories, including macroscopic changes, microscopic changes, organ weights and functional measures.

Reproductive effects in female rats noted in multiple studies, summarized above, included altered or abnormal estrous cycles, decreased ovarian weight, reduced numbers of corpora lutea, decreased implantation sites, litter sizes, and acceleration of sexual maturation.

With the exception of varying accelerated sexual maturation, all of these effects occurred at dose levels that also caused systemic toxicity. Systemic toxicity was typically evidenced by reduced body weight gain, reduced food consumption, altered hematological and serum chemistry values, and adrenal and liver hypertrophy. However, the female reproductive effects observed repeatedly following treatment with TPP are unlikely to be evidence of secondary results of systemic effects.

In reproductive studies conducted under various conditions of food restriction, rats have been repeatedly shown to be resistant to adverse reproductive effects unless there are marked effects upon body weight, typically to less than 70% of control body weight. For example, Chapin *et al*. (1993) used food restriction to control the body weights of adult Sprague-Dawley rats to 90%, 80%, and 70% of control (i.e., ad lib feeding) rat body weight. This resulted in reduced ovary weight, reduced corpora lutea in the ovary, and prolonged estrous cycle length *only* with body weight reduced to 70% of the control weight. There were no such effects at either 80% or 90% of control, and no statistically significant effects upon implants/dam (apparent reduction in implants only at 70% body weight, not statistically significant).

For comparison, female body weights at the start of mating were reduced in the one-generation gavage study to no less than 90% of the concurrent control at the top dose of 125 mg/kg/day, while in the two-generation dietary study, female body weights in the F0 and F1 females of the high dose (75 mg/kg/day) at the start of their first mating were 87% and 99% of the body weights of their concurrent controls. Similarly, Seki et al., (1997) restricted feed consumption for 13 weeks to 85%, 70%, and 55% of ad lib ingestion in Sprague-Dawley rats. In females, this resulted in body weight reductions of ca. 76%, 66%, and 53% of control; however, only the females with body weights reduced to 66% or less of concurrent control value displayed estrous cycle alterations and reduced ovary weights. Furthermore, in both of these studies specifically designed to better understand the

secondary effects of food restriction upon general function and reproduction, the gonad is relatively spared, i.e., the decrease in weight of the ovary is proportionally smaller than the degree of change to body weight. Thus, in female Sprague-Dawley rats limited to 66% control body weight, the ovary was 79% of concurrent control ovary weight (Chapin *et al.*, 1993a).

The acceleration of sexual maturation in female rats is opposite to the effect that would be produced secondary to reduced body weights or delayed pup growth in offspring. Food restriction during in utero and postnatal development resulting in as much as a 21% decrement in offspring weight on PND 21 had no effect upon timing of vaginal opening, while more severe restriction to 53% of concurrent control weight at weaning on PND 21 resulted in a subsequent delay in vaginal opening (32.9 days vs. 38.6 days). In contrast, females exposed to TPP in the two-generation dietary study and the female pubertal assay had an accelerated vaginal opening (29.1 days vs. 33.2 days, and 27.4 days vs. 32.4 days, respectively) with and without delayed postnatal growth.

4.11.5 Comparison with criteria

Results from reproduction studies, repeat-exposure studies, and mechanistic assays with TPP are evaluated together for a weight of evidence approach and compared to the criteria for the Classification and Labelling of this substance in the EU pursuant to REACH. Classification is made according to the criteria of the Dangerous Substances Directive (DSD) (67/548/EEC, as amended) and the Classification, Labelling and Packaging Directive (CLP) (1272/2008/EC).

Multiple study results indicate reproducible, consistent, and clear effects of TPP on the reproductive system in rats with the potential relevance of these effects to humans demonstrated by the estrogen-sensitive assays. The weight of evidence from these findings support a classification for TPP of:

- **Category 2: R60, May Impair Fertility, according to the Dangerous Substances Directive (DSD) and**
- **Category 1B, Presumed Reproductive Hazard to Humans, according to the Classification, Labelling and Packaging Directive (CLP).**

According to CLP, the classification of hazard class Reproductive Toxicity is differentiated into adverse effects on sexual function and fertility, and/or on development. Since adverse effects meeting the criteria of classification were only observed on sexual function and fertility following exposure to TPP, these endpoints are used for comparison to CLP classification criteria below. For comparison purposes, a CLP Category 1A, 1B, or 2 Reproductive Toxicant is equivalent to a DSD Category 1, 2, or 3 Reproductive Toxicant, respectively.

Criteria for Category 1: Known or presumed human reproductive toxicant

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

Criteria for Category 1A: Known human reproductive toxicant

The classification of a substance in this Category 1A is largely based on evidence from humans.

No human information is available for comparison of TPP with the subcategory 1A.

Criteria for Category 1B: Presumed human reproductive toxicant

The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Key considerations:

(a) Exposure to TPP in animal studies resulted in clear evidence of alterations to the onset of puberty, estrous cyclicity, perturbations to the reproductive system, and impairment of fertility. Although these findings were generally accompanied by reductions in body weight, no clear evidence was found that these effects could be solely attributed as secondary harm due to systemic toxicity.

Exposure to TPP in animal studies resulted in adverse effects on reproduction in male and female rats accompanied by evidence of generalized systemic toxicity at high dose levels. The systemic effects presented as reductions in body weight and body weight gain and were generally mild to moderate in females except where doses were sufficient to induce marked systemic toxicity (e.g. lethality, dramatic reduction in body weight, or coma). At the low to mid-range doses in these studies, reduced weight was often accompanied by a decline in food efficiency.

In published studies assessing the effects of food deprivation in rats, impacts on reproductive parameters were only observed in the presence of severe weight reduction (< 70% of control) and reductions in female body weight to 80% and 85% of control were shown to have no effect on reproductive performance or other reproductive parameters in rats (Carney *et al*, 2004; Chapin *et al*, 1993; Seki *et al*, 1997).

Given the observations that female body weight and body weight gain in the TPP studies were similar in magnitude to the mild to moderate effects observed in food deprivation described above, these observed effects on weight cannot account for the observed alterations in reproductive endpoints.

(b) This interpretation is supported in the Guidance on the Application of the CLP Criteria under section 3.7.2.2.1:

Classification in the presence of parental toxicity

Effects to be considered in the presence of marked systemic effects

In general all findings on reproductive toxicity should be considered for classification purposes irrespective of the level of parental toxicity. A comparison between the severity of the effects on fertility/development and the severity of other toxicological findings must be performed.

Fertility effects

Adverse effects on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) are not relevant for classification purposes. There is no established relationship between fertility effects and less marked systemic toxicity. Therefore it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity are not a secondary consequence of this toxicity. However, mating behaviour can be influenced by parental effects not directly related to reproduction (e.g. sedation, paralysis), and such effects on mating behaviour may not warrant classification.

At the highest dose levels evaluated, adverse reproductive effects were observed at doses that caused marked systemic toxicity (e.g. reduced body weight to < 70% of control) and these were determined to be irrelevant for classification purposes due to the severity of the overall toxicity. The middle doses also showed evidence of reproductive toxicity and these effects occurred at dose levels causing "less marked systemic toxicity" (e.g. reduced body weight 80% to 90% of control) and therefore cannot be assumed to be a secondary consequence of toxicity as stated in the guidance document.

(c) Mechanistic screening assays related to reproductive function indicate estrogenic activity.

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

Two uterotrophic assays were conducted on TPP as manufactured and purified. The interpretation of the laboratory for both studies was that TPP "demonstrated or mimicked biological activities consistent with agonism of natural estrogens".

Four female pubertal assays were conducted on TPP as manufactured and purified. 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.

TPP is considered a weak androgen receptor binder following an *in vitro* rat prostate androgen competitive binding assay and an estrogen receptor binder following an *in vitro* rat uterine estrogen receptor competitive binding assay.

Criteria for Category 2: Suspected human reproductive toxicant

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Given the large data set available for evaluation of the reproductive endpoint and given the overall quality of the dataset, it cannot be assumed that there are deficiencies that make the quality of evidence less convincing or that a Category 2 classification is warranted. The evidence becomes clear when evaluated as a weight of evidence approach because effects can be seen in a dose responsive, statistically significant, and reproducible manner across multiple study designs. These findings include reduced mean live litter size, altered estrous cyclicity, reduced ovary weights and ovary histology. Increased uterine weight was observed in the uterotrophic assay, as was accelerated sexual maturation in immature animals in the female pubertal assay. Alterations in male reproductive parameters, including effects on multiple accessory organs, sperm production, and transport, were also identified in all studies where evaluated but concurrent with significant changes to general health. Marked systemic toxicity as explained in CLP guidance documents is *lethality, dramatic reduction in absolute body weight, coma.* Therefore, the reductions in parental body weight are insufficient to be the primary cause for the observed changes in reproductive endpoints in females as several effects occur at body weight reductions within 80% of control.

4.11.6 Conclusions on classification and labelling

Multiple test results indicate clear, reproducible and consistent adverse effects of TPP on the reproductive system in rats with the potential for relevance of these effects to humans. The weight of evidence from these findings supports a classification for TPP of:

- Category 2: R60, May Impair Fertility, according to the Dangerous Substances Directive (DSD) or
- Category 1B, Presumed Reproductive Hazard to Humans, according to the Classification, Labeling and Packaging Regulation (CLP).

No classification is proposed for developmental effects.

Reproductive toxicity

Summary of the Dossier submitter's proposal

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

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

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

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

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

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

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

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

The findings include reduced mean live litter size, altered oestrous cyclicity, reduced ovary weights and ovary histology. Increased uterine weight was observed in the uterotrophic assay,

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

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

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

Comments received during public consultation

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

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

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

Table 1. Estimation of ED_{10} values using linear extrapolation for ovary weight in rats exposed to TPP

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

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

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

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

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

Assessment and comparison with the classification criteria

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

Adverse effects on sexual function and fertility

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

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

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

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

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

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

Parameter F0 females and offspring	**Dose Level (mg/kg bw/day)**			
	0	**1.5**	**15**	**75**
Mean absolute organ weights and microscopic findings (incidences)				
Mean terminal body weight (g)	325	323	321	286** (↓12%)
Mean body weight (g) - initiation of mating	293	290	284	256** (↓12.6%)
Mean body weight gain (g) – initiation of mating	126	123	117	89** (↓29.4%)
Mean ovaries weight (g)	0.1202	0.1210	0.1142	0.0846** (↓30%)
Ovaries – decreased presence of corpora lutea (5 or less)	1/30	0/27	0/30	6/28* (↓18%)
Oestrous cycle length (days) (historical control range: 3.6 – 5.8 days)	4.3	4.3	4.5	5.4**
Persistent oestrus (>3 consecutive days)	1/30	0/30	0/30	0/30
Persistent diestrus (>4 consecutive days)	0/30	0/30	6/30	12/30
Number of implantation sites (measured in F0 only) (historical control range: 12.6 – 17.0)	15.0	14.8	14.7	13.2* (↓12%)
Number born (historical control range: 13.0 – 16.6)	14.0	14.1	14.0	12.5
Live litter size (historical control range: 12.6 - 16.4)	13.8	13.9	13.7	12.2
Pup weight (M/F) – PND 1	7.5/7.0	7.5/7.0	7.3/6.9	7.2/6.8
Pup weight (M/F) – PND 4	10.4/9.8	10.2/9.6	10.0/9.6	9.6/9.0
Pup weight (M/F) – PND 7	16.5/15.7	16.1/15.3	15.1*/14.4**	14.3**/13.5**
Pup weight (M/F) – PND 14	30.1/29.2	30.1/29.1	28.6/27.3	22.9**/21.8**
Pup weight (M/F) – PND 21	50.7/49.3	49.1/46.8	47.0/45.0*	36.4**/34.4**

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

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

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

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

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

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

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

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

Parameter F1 Males	**Dose Level (mg/kg bw/day)**			
	0	**1.5**	**15**	**75**
Mean organ absolute weights and microscopic findings (incidence)				
Mean terminal body weight (g)	791	814	754	566** (↓28.4%)
Mean body weight (g) - initiation of mating	543	545	536	449** (↓17.3%)
Mean testes weight (g) left	1.87	1.94	1.94	1.74
Mean testes weight (g) right	1.93	1.96	1.88	1.72** (↓11%)
Mean epididymis weight (g) left	0.67	0.73	0.75* (↑12%)	0.65
Mean epididymis weight (g) right	0.76	0.80	0.77	0.68** (↓10.5%)
Epididymis sperm concentration (x106/g) left	310.1	339.4	350.2	320.5
Mean cauda epididymis weight (g) left	0.3028	0.3362	0.3391* (↑12%)	0.2740
Mean cauda epididymis weight (g) right	0.3349	0.3588	0.3372	0.2879** (↓14%)
Mean cauda epididymis weight relative to body weight (g/100g) left	0.039	0.042	0.046**	0.049** (↑25%)
Mean cauda epididymis weight relative to body weight (g/100g) right	0.043	0.045	0.045	0.052** (↑21%)
Mean cauda epididymis weight relative to brain weight (g/100g) left	13.751	15.663* (↑14%)	15.825** (↑15%)	13.116
Mean cauda epididymis weight relative to brain weight (g/100g) right	15.253	16.714	15.720	13.815
Mean prostate weight (g)	1.06	1.07	1.06	0.92* (↓13%)
Mean prostate weight relative to brain weight (g/100g)	48.133	49.916	49.262	44.003
Mean seminal vesicle weight (g)	2.19	2.26	2.2	1.81** (↓17)
Mean seminal weight relative to body weight (g/100g) right	0.28	0.284	0.296	0.32** (↑14%)

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

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

controls but values in all groups were low as a consequence of the age of animals at the second mating, and hence data for this second mating cannot be considered as robust. Gestation length was unaffected by treatment in both the first and second mating.

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

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

It is noted that among the three generations of litters observed in the two-generation study, in one generation (F2a) there was a reduction of litter size at 75 mg/kg bw/day, and there was a decrease in the mean number of implantation sites in F0 females at 75 mg/kg bw/day. These values were well within the historical control range (12.6-17.00 for implantation sites and 12.6 – 16.4 for live litter size). Thus the effect of TPP on the litter size in 1 out of 3 generations of litters observed only at 75 mg/kg bw/day, which also caused clear maternal toxicity, does not provide a strong presumption that the substance interferes with fertility.

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

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

Table 6. One-generation study - findings in parental animals

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

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

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

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

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

The adverse effect on fertility in the adult rats was accompanied by adverse microscopic changes in both male and female reproductive organs, adverse effects on female cyclicity, and a reduction in epididymal sperm concentration (effects described below). The reduction in fertility and effects of reproductive organs occurred at doses that also induced other toxic effects, including reduced body weight gain and food consumption and changes in the adrenals, kidneys and liver. However, this toxicity to non-reproductive organs was insufficient to deem the reproductive findings as secondary non-specific effects.

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

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

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

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

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

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

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

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

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

90-day study in rats with oral exposure in diet (Vogin, 1970a):
Study design: FDRL rats (20/sex/group), 90-day treatment via diet containing 0, 0.05, 0.2 and 0.4% of TPP (equivalent to 0, 25, 100 and 200 mg/kg bw/day) 7 days/week. Test material: Phenol, dodecyl (CAS 27193-86-8).

No deaths occurred and no clinical observations of toxicity were observed during the study period. Weight gain and food utilisation efficiency was reduced at 200 mg/kg bw/day in males (81.6% of control males' body weight) and females (89% of control females' body weight). Mean absolute and relative testes weights were reduced in males at 200 mg/kg bw/day with testicular hypospermia observed in 6 out of 20 animals. Additionally, liver weights were increased among either sex at 200 mg/kg bw/day. No additional histopathological effects were noted in this study. A NOAEL of 100 mg/kg bw/day was assigned for general toxicity and effects on the male reproductive tract.
The results of this study indicate that the effect of the test material on the male reproductive tract at the highest dose level of 200 mg/kg bw/day was associated with reduced weight gain.

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

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

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

Summary of effects on female fertility:

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

In the one-generation study by Knapp (2006), mean absolute ovarian weight was significantly reduced in females at 25 and 125 mg/kg bw/day (87% and 70%, respectively, of control values). Microscopic evaluation of ovaries revealed an increase in ovarian cysts (in 15/30 animals vs. 4/30 in controls) and decreased corpora lutea (in 18/30 animals vs. 4/30 in controls) at 125 mg/kg bw/day. Uterine weight was unaffected, although this measure may not have been valid due to differences between exposure groups in proportions of rats that had produced litters. Microscopically, an increase in endometrial gland cysts (8/30 animals vs. 1/30 in controls) was detected at 125 mg/kg bw/day. At 125 mg/kg bw/day, a disproportionate number of females, many of which had mated but did not show evidence of pregnancy (implantation sites at necropsy), were in oestrus at termination (16/30 vs. 3/30 in controls). This finding mirrored changes to oestrous cyclicity detected during weeks 7-10 of exposure. At the mid and high dose, oestrous cycle length increased (4.9 and 5.2 days, respectively, vs. 4.4 days in controls). In the

high dose group, 6/30 females and 16/30 females displayed persistent oestrus or diestrus, respectively, and 6/30 females were essentially acyclic (vs. 0/30, 2/30, and 0/30 for each endpoint, respectively, in controls).

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

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

Summary of effects on male fertility:

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

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

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

In the one-generation study (Knapp, 2006) at 25 mg/kg bw/day, there was a significant

decrease in the mean cauda epididymides absolute weight compared to controls, which was also significantly reduced relative to brain weight. Histopathological findings at this dose level included a significant increase in the number of animals with decreased secretions in the coagulating and prostate glands compared to controls.

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

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

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

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

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

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

Several publications which have examined the relationship between body weight changes and male reproductive organ weight changes in the rat (Scharer, 1977; Chapin *et al*., 1993; Levin *et al*., 1993; Keenan *et al*., 1994; Seki *et al*., 1997; Odum *et al*., 2001; Marty *et al*., 2003; Carney *et al*., 2004; Terry *et al*., 2005; Laws *et al*., 2007) have been summarized in OECD draft guidance document 151 (http://www.oecd.org/env/ehs/testing/GD%20151_Oct%202012_clean2.pdf). These studies showed that reductions in the weights of testes and epididymides were usually smaller than reductions in body weight. A 15% body weight reduction was correlated with a testes and epididymides weights reduction of 2-12%; a 40% body weight reduction resulted in testes and epididymides weights being reduced by 24%. Prostate and seminal vesicle weight varied more with body weight. At 10% body weight reduction, prostate and seminal vesicle weights were reduced by 0-20% and at 40% body weight reduction, prostate and seminal vesicle weights were reduced by 20-45%.

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

Mechanistic Studies Related to Reproductive Toxicity

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

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

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

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

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

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

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

Summary of effects in the uterotrophic bioassays:

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

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

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

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

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

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

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

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

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

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

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

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

Study design: Crl:CD (SD) immature female rats were exposed to 5, 20, or 60 mg/kg bw/day of

calcium salt of TPP once daily for 20 consecutive days (PND 22-41) by oral gavage.

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

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

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

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

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

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

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

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

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

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

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

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

the presence of the test substance.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Study design: Test material: EC No. 430-180-1 (26 wt% TPP); SD rat, 28 animals per dose and

sex, exposure by oral gavage, doses of 0, 5, 30 & 150 mg/kg bw/day (0, 1.3, 7.8, 39 mg/kg bw/day of TPP); Exposure: F0 dosed for a minimum of 10 weeks prior to mating and then to dose-matched mating pairs throughout the mating, gestation and lactation phases. F1 offspring from each dose group were selected to proceed into the main study (at weaning). F1 dosed for a minimum of 10 weeks. Following this dosing period, the main study F1 animals were paired within dosing groups and subsequently dosed throughout mating, gestation and lactation to yield the F2 litters.

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

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

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

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

Since the detailed composition of the mixtures used in the above studies and the purpose of investigating reproductive toxicity of these mixtures are unknown, RAC is of the opinion that the results of these studies have very limited relevance for classification of TPP for reproductive toxicity, therefore these studies will not be considered for justification of harmonized classification for TPP or for justification of SCL for this substance.

Developmental toxicity

Schroeder, 1987: Prenatal developmental toxicity study in rats (OECD TG 414, GLP compliant), key study
Study design: SD female rats were exposed once daily to 0, 20, 100 or 300 mg/kg bw/day of TPP by oral gavage during days 6 - 15 of gestation; Foetuses were evaluated for external, visceral, and skeletal alterations. Due to excessive mortality, dams in an additional group (500 mg/kg bw/day) were sacrificed on day 20 of gestation. Their uterine contents were examined.

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

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

In the two-generation study (Edwards *et al*., 2012; for details see 'Adverse effects on sexual function and fertility'), the timing of sexual maturation was significantly altered in both the male

and female offspring of the first generation in the 75 mg/kg bw/day exposure group. At 75 mg/kg bw/day, pup body weights were significantly reduced in the F2 litters on PND 1 and PND 7-21 and in F2a litters on PND 14 and 21 compared to controls. However, no reduction in F2 and F2a pup body weight was observed at15 mg/kg bw/day.

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

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

In the one-generation study (Knapp, 2006; for details see 'Adverse effects on sexual function and fertility'), pups with potential exposure during gestation and lactation that were maintained in the study after weaning without post-weaning dosing, had unaffected sexual maturation in the 5 and 25 mg/kg bw/day groups (no statistical evaluation of pups from the 125 mg/kg bw/day group due to insufficient litters). Offspring at 25 mg/kg bw/day showed statistically significantly reduced body weight gain compared to controls between PND 4 and 21. Pup body weight gain was not statistically evaluated for the 125 mg/kg bw/day dose group due to the small sample size.

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

Observations	**Time point**	**Sex**	**Dose level** (mg/kg bw/day)			
			0	**5**	**25**	**125**
Signs of toxicity		M/F	-	-	✔	✔
Litter size (#)	Day 0	M/F	13.3	14.0	12.4	2.3**
Viability (%)	Day 0	M/F	96.6	98.7	93.7	55.6
	Day 0-1	M/F	99.7	98.7	100	100
	Day 1-4	M/F	99.3	95.6	99.4	100
	Day 4-21	M/F	98.2	98.9	98.4	100
Pup weight(g)	Day 1	M	7.1	7.1	7.2	7.9
		F	6.6	6.7	6.8	8.0
	Day 4	M	9.6	9.9	9.6	10.8
		F	9.1	9.3	9.1	11.1
	Day 7	M	15.9	16.1	14.7	14.1

		F	14.6	15.3	13.5	16.9
	Day 14	M	33.0	33.5	29.9**	22.5
		F	31.2	32.3	28.0**	29.3
	Day 21	M	52.5	53.0	47.5**	34.5
		F	49.4	50.8	44.8**	46.1
Balano-preputial separation (day)		M	43.2	42.9	44.6*	47.5
Balano-preputial separation (g)			230.1	226.0	229.1	205.7
Vaginal patency (day)		F	33.0	32.8	33.5	32.5
Vaginal patency (g)			115.1	116.0	110.2	110.6

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

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

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

Summary of the classification justification

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

Considering these criteria, classification as Repr. 1B; H360F (CLP) (Repr Cat. 2; R60, DSD) is

justified for TPP based on the following effects observed in experimental studies:

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

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

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

Calculation of a concentration limit for reproductive toxicity:

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

Determination of the ED_{10} using the available data

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Modifying factors

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

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

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

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

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

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

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

level of 100 mg/kg bw/day, thus higher than the lower limit of 4 mg/kg bw/day and lower than 400 mg/kg bw/day, which is the upper limit of the medium potency group.

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

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

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

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

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

Conclusion

Conclusion on classification:

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

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

Conclusion on concentration limits:

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

4.12 Other effects

No data are available.

4.12.1 Non-human information

No data are available.

4.12.1.1 Neurotoxicity

No data are available.

4.12.1.2 Immunotoxicity

No data are available.

4.12.1.3 Specific investigations: other studies

No data are available.

4.12.1.4 Human information

No data are available.

4.12.2 Summary and discussion

No data are available.

4.12.3 Comparison with criteria

Not applicable.

4.12.4 Conclusions on classification and labelling

No classification is proposed.

5 ENVIRONMENTAL HAZARD ASSESSMENT

No proposal for environmental hazard classification is requested. Section 5 of this CLH report is retained per the template only to fulfil accordance with the CLH requirements.

5.1 Degradation

No proposal for classification is requested.

Table 31: Summary of relevant information on degradation

Method	Results	Remarks	Reference

Not applicable	-	-	-

5.1.1 Stability

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

5.1.2.3 Simulation tests

5.1.3 Summary and discussion of degradation

5.2 Environmental distribution

No proposal for classification is requested.

5.2.1 Adsorption/Desorption

5.2.2 Volatilisation

5.2.3 Distribution modelling

5.3 Aquatic Bioaccumulation

No proposal for classification is requested.

Table 32: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference

Not applicable	-	-	-

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

5.3.1.2 Measured bioaccumulation data

5.3.2 Summary and discussion of aquatic bioaccumulation

5.4 Aquatic toxicity

No proposal for classification is requested.

Table 33: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
Not applicable	-	-	-

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

5.4.1.2 Long-term toxicity to fish

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

5.4.2.2 Long-term toxicity to aquatic invertebrates

5.4.3 Algae and aquatic plants

5.4.4 Other aquatic organisms (including sediment)

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

No proposal for classification is requested.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

No proposal for classification is requested.

6 OTHER INFORMATION

Not applicable.

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8 ANNEXES

No annexes are included.