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-0000003060-91-04/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:

Phenol, dodecyl-, branched

EC Number: **310-154-3**

CAS Number: **121158-58-5**

Index Number: **NA**

Dossier submitter: SI Group-UK, Ltd

Version number: 4 **Date: 10th OCTOBER 2012**

CONTENTS

Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Phenol, dodecyl-, branched.

Table 1: Substance identity

Substance name:	Phenol, dodecyl-, branched [Tetrapropenylphenol (TPP)]
EC number:	310-154-3
CAS number:	121158-58-5
Annex VI Index number:	Not listed in Annex VI of Regulation 1272/2008.
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 currently listed	Not currently listed
Current proposal for consideration by RAC	Skin Irrit. 2 H315: Causes skin irritation Eye Irrit. 2 H319: Causes serious eye irritation Repr. 2 H361f: Suspected of damaging fertility Aquatic Chronic category 1:H410: Very toxic to aquatic life with long lasting effects [M-factor 10] Aquatic Acute Category 1; H400: Very toxic to aquatic life [M-factor 1]	R36/38 'Irritating to eyes and skin' R50/53 'Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment' Repr Cat 3: R62 Possible risk of impaired fertility Concentration limits: N; R50-53: Cn ≥ 25 % N; R51-53: 2.5% ≤ Cn < 25% R52-53: 0.25 % ≤ Cn < 2.5 %
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Hazard statements: Skin Irrit. 2 H315: Causes skin irritation	R36/38 'Irritating to eyes and skin' R50/53 'Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic

	Eye Irrit. 2 H319: Causes serious eye irritation Repr. 2 H361f: Suspected of damaging fertility Aquatic Chronic category 1:H410: Very toxic to aquatic life with long lasting effects [M-factor 10] Aquatic Acute Category 1; H400: Very toxic to aquatic life [M-factor 1]	environment' Repr Cat.3 R62 Possible risk of impaired fertility Concentration limits: N; R50-53: Cn ≥ 25 % N; R51-53: 2.5% ≤ Cn < 25% R52-53: 0.25 % ≤ Cn < 2.5 %

1.3 Proposed harmonised classification and labelling based on CLP Regulation

For a harmonised classification in accordance with CLP requirements, none of the physicochemical properties of phenol, dodecyl-, branched lead to classification. Specifically, the substance is not oxidising, flammable or explosive. With respect to mammalian toxicity and its impact on human health, the properties of the substance indicate the need to classify phenol, dodecyl-, branched with the Signal Word 'WARNING' and the Hazard Statements H315 (causes skin irritation), H319 (causes serious eye irritation) and H361f (suspected of damaging fertility). For environmental hazards the relevant CLP classification is H410 (Very toxic to aquatic life with long lasting effects). For the aquatic environmental hazards based on the results of algal toxicity studies, phenol, dodecyl-, branched should bear the signal word 'WARNING'; the aquatic acute and chronic Category 1 classification with associated Hazard Statements H400 (Very toxic to aquatic life) and H410 (Very toxic to aquatic life with long lasting effects).

Table 3: Summary of 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	Skin Irrit. 2 (Hazard	Not applicable	Not classified	

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification	Reason for no classification
		statement H315: Causes skin irritation)			
3.3.	Serious eye damage / eye irritation	Eye Irrit. 2 (Hazard statement: H319: Causes serious eye irritation.)	Not applicable	Not classified	
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. 2 (Hazard statement: H361f: Suspected of damaging fertility	GCL of 3.0% applies under CLP	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	Aquatic Acute 1; H400: Very toxic to aquatic life Aquatic Chronic 1 (Hazard statement: H410: Very toxic to aquatic life with long lasting effects.)	M-factor 1[acute] M-factor = 10 [chronic]	Not classified	
5.1.	Hazardous to the ozone layer	None	Not applicable	Not classified	Data lacking

Labelling:

Signal word:

Warning

Hazard pictograms:

GHS08: health hazard

GHS09: environment

GHS07: exclamation mark

Hazard statements:

H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
H361f:	Suspected of damaging fertility
H410:	Very toxic to aquatic life with long lasting effects.

Precautionary statements:

P201:	Obtain special instructions before use.
P202:	Do not handle until all safety precautions have been read and understood.
P264:	Wash thoroughly after handling.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P281:	Use personal protective equipment as required.
P273:	Avoid release to the environment.
P302+P352:	IF ON SKIN: Wash with plenty of soap and water.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308+P313:	IF exposed or concerned: Get medical advice/attention.
P321:	Specific treatment (see... on this label).
P332+P313:	If skin irritation occurs: Get medical advice/attention.
P362:	Take off contaminated clothing and wash before reuse.
P391:	Collect spillage.
P405:	Store locked up.

P501: Dispose of contents/container to special waste

Proposed notes assigned to an entry:

Not applicable.

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification	Reason for no classification
Explosiveness	None	Not applicable	NA	Conclusive but not sufficient for classification
Oxidising properties	None	Not applicable	NA	Conclusive but not sufficient for classification
Flammability	None	Not applicable	NA	Conclusive but not sufficient for classification
Thermal stability	None	Not applicable	NA	Conclusive but not sufficient for classification
Acute toxicity	None	Not applicable	NA	Conclusive but not sufficient for classification
Acute toxicity-irreversible damage after single exposure	None	Not applicable	NA	Conclusive but not sufficient for classification
Repeated dose toxicity	None	Not applicable	NA	Conclusive but not sufficient for classification
Irritation / Corrosion	Xi, R36/38 Irritant; Irritating to eyes and skin	Not applicable	NA	
Sensitisation	None	Not applicable	NA	Conclusive but not sufficient for classification
Carcinogenicity	Not classified	Not applicable	NA	Insufficient data
Mutagenicity-Genetic toxicity	None	Not applicable	NA	Conclusive but not sufficient for classification
Toxicity to reproduction -fertility	Repr. Cat. 3; R62 Possible risk of impaired fertility	Not applicable	NA	
Toxicity to reproduction-development	None	Not applicable	NA	Conclusive but not sufficient for classification
Toxicity to reproduction-breastfed babies. Effects on or via lactation	Not classified	Not applicable	NA	Insufficient data
Environment	N; R50/53 Dangerous for the environment; Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.	Concentration limits: N; R50-53: Cn ≥ 25 % N; R51-53: 2.5% ≤ Cn < 25% R52-53: 0.25 % ≤ Cn < 2.5 %	NA	

Labelling:

Indication of danger:

N	Dangerous for the environment
Xn	Harmful
Xi	Irritant

R-phrases:

R36/38	Irritating to eyes and skin
R50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
R62	Possible risk of impaired fertility

S-phrases:

S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection
S60	This material and its container must be disposed of as hazardous waste
S61	Avoid release to the environment. Refer to special instructions/safety data sheets

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

The substance phenol, dodecyl-, branched is not listed on Annex VI of the CLP Regulation (EC) No. 1272/2008. The substance therefore has no formally agreed classification status in the EU.

2.2 Short summary of the scientific justification for the CLH proposal

The proposal for the harmonised classification and labelling of phenol, dodecyl-, branched is on the basis of the data presented in this dossier and which was also provided to ECHA in the Registration Dossier as part of the Joint Registration of this substance for the 2010 REACH deadline.

None of the physicochemical properties of phenol, dodecyl-, branched require classification according to the criteria applied under the Classification, Labelling and Packaging Regulation (CLP) Regulation. Specifically, phenol, dodecyl-, branched does not meet any of the classification criteria to be considered explosive, an oxidising material, a flammable solid or a self-heating substance.

Although the absorption, distribution, metabolism and excretion of phenol, dodecyl-, branched have not been directly studied *in vivo*, the available information indicates that it will cross physiological barriers and consequently become systemically available. Excretion via tissue elimination does

occur fairly rapidly but the lipophilicity of phenol, dodecyl-, branched means that tissue retention can also occur and such retention may be associated with long term tissue effects.

In experimental species, phenol, dodecyl-, branched is not acutely toxic via the oral or dermal routes. As a consequence of the low toxicity and low vapour pressure of the substance, low toxicity is also anticipated via the inhalation route. Phenol, dodecyl-, branched is irritating to skin and eyes but showed no indications of corrosivity. Tests for dermal sensitisation gave no evidence of delayed contact hypersensitivity potential.

No findings indicating any STOT-SE concerns were reported following administration by oral or dermal routes. A short-term (sub-acute) repeated dose toxicity study performed in the rat showed microscopic changes in male reproductive organs and some organ weight changes at higher dose levels (180 or 300 mg/kg bw/d) and microscopic findings of thyroid follicular cell hypertrophy in one male. Findings are considered likely to be secondary to marked bodyweight effects observed at these dose levels. A NOAEL of 60 mg/kg bw/d was determined for this study.

Sub-chronic repeated dose toxicity data are available for rats, in the form of two 90-day dietary studies. The NOAEL for one study was 25 mg/kg bw/d, with bodyweight and testicular effects at higher dose levels of 100 and 200 mg/kg bw/d. In a second study, bodyweight effects were apparent at all dose levels (≥50 mg/kg bw/d); effects on male reproductive tract organ weights were observed at dose levels of ≥100 mg/kg bw/d, with histopathological effects apparent at dose levels of ≥150 mg/kg bw/d. A thirteen week dietary dog study did not demonstrate any toxicity, resulting in a NOAEL of 200 mg/kg bw/d (i.e. the highest dose administered). The sub-acute and sub-chronic repeated exposure toxicity studies do not therefore provide a basis to support classification for repeated exposure toxicity (STOT-RE criteria).

Based on results obtained in an appropriately designed battery of mutagenicity studies, phenol, dodecyl-, branched is not considered to be genotoxic. Negative results in the *in vitro* mutagenicity studies were confirmed by the negative results of an *in vivo* micronucleus induction assay in rat erythrocytes. On the basis of this data, phenol, dodecyl-, branched does not require classification for mutagenicity.

No data are available for carcinogenicity; however the results of the genotoxicity studies do not indicate any carcinogenic potential for phenol, dodecyl-, branched. On the basis of this data, phenol, dodecyl-, branched does not require classification for carcinogenicity.

The reproductive toxicity of phenol, dodecyl-, branched has been investigated extensively. In a one-generation study using garage administration, marked effects on fertility associated with significant bodyweight effects were observed at the highest dose level of 125 mg/kg bw/d. Effects on male reproductive tract organ weights and histopathology (reduced secretory activity of the prostate, seminal vesicles and coagulating gland) were seen at 25 and 125 mg/kg bw/d. Similar effects on fertility were not apparent in a two-generation dietary toxicity performed at dose levels of up to 75 mg/kg bw/d and sufficient to cause bodyweight effects of a similar magnitude. Sperm analysis performed in the one-generation study revealed a significantly reduced epididymal sperm concentration in males at 125 mg/kg bw/d. Mean testicular sperm numbers and sperm production rates, motility, progressive motility and morphology were unaffected by treatment; similar effects were apparent at 75 mg/kg bw/d in the two-generation toxicity study. Vaginal patency was attained at an earlier age and associated bodyweight at 75 mg/kg bw/d in the two-generation toxicity study. Balano-preputial separation was significantly delayed at this dose level but was considered to be secondary to effects on offspring bodyweight. Persistent dioestrus was also observed at 125 mg/kg bw/d in the one-generation study and at 75 mg/kg bw/d in the two-generation study.

A developmental toxicity study performed in the rat using phenol, dodecyl-, branched is available. An increased incidence of malformations and variations was seen in this study; elevated incidences of external findings (cleft palate, ectrodactyly, brachydactyly) and skeletal findings (wavy ribs, misshapen long bones) were observed at the highest dose level of 300 mg/kg bw/d. Findings were associated with maternal toxicity at this dose level and with marked toxicity in individual dams.

On the basis of findings in the reproductive and developmental toxicity it is proposed to classify phenol, dodecyl-, branched as Reproductive Category 3 according to Directive 67/548/EEC (R62) 'Possible risk of impaired fertility' and according to the CLP Regulation (EC) No 1272/2008 as classified as Repro Category 2; H361f: Suspected of damaging fertility.

Several studies (both acute and long-term) were available on aquatic organisms (fish, *Daphnia*, sediment-dwelling organisms, algae and higher plants) for phenol, dodecyl-, branched.

The 96-hour median lethal concentration (LC_{50}) of the water accommodated fraction (WAF) of phenol, dodecyl-, branched to fathead minnows was 40 mg/L (expressed as the nominal amount of test substance used to prepare the WAF) with a 95% confidence interval of 25 to 50 mg/L. The estimated no observed effect concentration (NOEC) was 25 mg/L. The LC_{50} for freshwater fish was determined as 40 mg/L.

The short term toxicity endpoints for aquatic invertebrates are:

EC_{50}/LC_{50} for freshwater invertebrates: 0.037 mg/L

EC_{50}/LC_{50} for marine water invertebrates: 0.58 mg/L

The long term toxicity endpoints for aquatic invertebrates are:

EC_{10}/LC_{10} or NOEC for freshwater invertebrates: 0.0037 mg/L (on the basis that at this test concentration there were no significant mortalities (immobilisation) observed in the parental generation (P_1) and that there were no significant difference ($p \leq 0.05$) between the solvent control and the 0.0037 mg/L test group in terms of numbers of live young produced per adult by Day 21).

Endpoints for algae and aquatic plants included:

EC_{50}/LC_{50} for freshwater algae: 0.36 mg/L

EC_{10}/LC_{10} or NOEC for freshwater algae: 0.07 mg/L

The acute and chronic endpoints driving the environmental classification were observed in a laboratory study with phenol, dodecyl-, branched and the unicellular green alga *Pseudokirchneriella subcapitata* (72h E_rC_{50} = 0.36 mg/L) and a 21-day *Daphnia magna* reproduction test (21-day NOEC = 0.0037 mg/L). Based on the results of these studies, acute and chronic M-factors of 1 and 10 respectively are appropriate for phenol, dodecyl-, branched.

In toxicity studies performed using algae and *Daphnia*, EC_{50} values below 1 mg/L were obtained (EC_{50}/LC_{50} for freshwater algae: 0.36 mg/L, NOEC for *Daphnia magna* 0.0037 mg/L). In addition, phenol, dodecyl-, branched is not readily biodegradable. Based on these findings phenol, dodecyl-, branched should be classified as:

N (R50/53) according to Directive 67/548/EEC

Aquatic Chronic 1; H410 (Very toxic to aquatic life with long lasting effects); Aquatic Acute 1; H400 (Very toxic to aquatic life) according to Regulation (EC) no 1272/2008.

As a result of the R50-53 classification when phenol, dodecyl-, branched is used in preparations, the concentration limits and the resulting classifications based on Annex III, Table 1b of Directive 2006/8/EC are applicable:

Concentration Limits according to Annex III Table 1b of Directive 2006/8/EC, based on an LC50 value of 0.36 mg/L.

N; R50-53: Cn ≥ 25 %
N; R51-53: 2.5% ≤ Cn < 25%
R52-53: 0.25 % ≤ Cn < 2.5 %

2.3 Current harmonised classification and labelling

There is no current harmonised classification in Annex VI of CLP.

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

Phenol, dodecyl-, branched 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

Phenol, dodecyl-, branched is not currently listed in Annex VI, Table 3.2 of the CLP Regulation.

2.4 Current self-classification and labelling

CAS 121158-58-5 (EC 310-154-3) has been notified and is listed on the CLP inventory with classification as Skin Irrit. 2 (H315) or Skin Corr 1A (H314); Eye Irrit. 2 (H319) or Eye Dam. 1 (H318); Repr. 2 (H361) or Repr 1B (H360) Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410).

EC 310-154-3 has been notified under REACH and two registration dossiers are disseminated. The classification proposed in one REACH registration dossier is Skin Irrit. 2 (H315), Eye Irrit. 2 (H319), Repr 2 (H361) and Aquatic Chronic 1 (H410). The classification proposed in the second REACH registration dossier is Skin Irrit. 2 (H315), Eye Irrit. 2 (H319), Repr 2 (H361), Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410).

The current self-classification of phenol, dodecyl-, branched under Directive 67/548/EEC and according to Regulation (EC) No. 1272/2008 is as described above.

No classification for phenol, dodecyl-, branched on the basis of its physicochemical properties is justified. No classification for acute toxicity is appropriate on the basis of the available data: no specific target organ toxicity following single exposure (STOT-SE) indications were evident in the database. Phenol, dodecyl-, branched is classified for skin irritation (H315) and eye irritation (H319). There is no evidence for skin sensitisation. Repeated dose toxicity data do not support classification for specific target organ toxicity following repeated exposure (STOT-RE).

No evidence of genotoxicity was apparent in an appropriate battery of studies *in vitro* and *in vivo*; there is therefore no evidence for genotoxicity or carcinogenicity. Classification as H361f-Suspected of damaging fertility is on the basis of the available data.

Self-classification according to the criteria of the CLP Regulation also includes the perceived environmental risk and based on the results of studies in algae, phenol, dodecyl-, branched is

classified as Aquatic Acute Cat 1, Aquatic Chronic Cat 1, H400: Very Toxic to aquatic life; H410: Very Toxic to aquatic life with long lasting effects.

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

Labelling:

Signal word: Warning

Hazard pictogram:

GHS08: health hazard

GHS09: environment

GHS07: exclamation mark

Hazard statements:

H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
H361f:	Suspected of damaging fertility
H410:	Very toxic to aquatic life with long lasting effects.

Precautionary statements:

P201:	Obtain special instructions before use.
P202:	Do not handle until all safety precautions have been read and understood.
P264:	Wash thoroughly after handling.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P281:	Use personal protective equipment as required.
P273:	Avoid release to the environment.
P302+P352:	IF ON SKIN: Wash with plenty of soap and water.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308+P313:	IF exposed or concerned: Get medical advice/attention.
P321:	Specific treatment (see... on this label).

P332+P313:	If skin irritation occurs: Get medical advice/attention.
P362:	Take off contaminated clothing and wash before reuse.
P391:	Collect spillage.
P405:	Store locked up.
P501:	Dispose of contents/container to special waste

2.4.2 Current self-classification and labelling based on DSD criteria

(R36/38):	Irritating to eyes and skin
(R62):	Possible risk of impaired fertility
(R50/53):	Dangerous for the environment; Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Indication of danger:

N:	Dangerous for the environment
Xn:	Harmful
Xi:	Irritant

R-phrases:

R36/38-irritating to eyes and skin

R50/53-very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

R62-possible risk of impaired fertility

S-phrases:

S26-in case of contact with eyes, rinse immediately with plenty of water and seek medical advice

S36/37/39-wear suitable protective clothing, gloves and eye/face protection

S60-this material and its container must be disposed of as hazardous waste

S61-avoid release to the environment. refer to special instructions/safety data sheets

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The CLP inventory contains a number of different proposals for the classification of EC 310-154-3 in the areas of skin irritation/corrosivity, eye irritation, reproductive toxicity and aquatic toxicity. There is a therefore a need for harmonisation of the non-CMR endpoints in order to ensure adequate risk management throughout the European Community; therefore consideration of the hazard endpoints other than those referred to in Article 36(1) of the CLP Regulation is justified.

RAC general comment

Substance identity:

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

Hazard classes:

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

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

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

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)

Commercial material is produced via the alkylation of phenol with propylene tetramer (CAS 6842-15-5). Tetrapropenylphenol is broadly used as an equivalent name for the substance reflecting the respective process of synthesis and its complex composition. The equivalent use of these names has been recognized in several previous assessment reports including the OECD HPV program:

(http://webnet.oecd.org/hpv/ui/SIDS_Details.aspx?id=2600a34f-69ca-4932-803c-86db6f1f5eaa)

and the UK environmental risk assessment:

(http://publications.environment-agency.gov.uk/PDF/SCHO0607BMVN-E-E.pdf)

The UK assessment notes that '*the term tetrapropenyl represents a large number of highly branched isomeric alkyl olefins ranging from* $C_{10}H_{20}$ *to* $C_{15}H_{3}0$*. Therefore, the chemical name tetrapropenylphenol represents both the presence of branched alkyl groups that may be located at either the 2-(ortho), 3-(meta), or 4-(para) position on the phenyl ring and a range of alkyl chain lengths. The predominant nominal* $C_{12}H_{25}$ *side chain alkyl group has a typical continuous carbon chain length of eight carbons with four methyl branches'*.

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
Common name:	Tetrapropenyl phenol (TPP)
IUPAC name:	Phenol, alkyl branched (species comprising decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, substituents)
CLP Annex VI Index number:	Not listed in DSD Annex or CLP Annex VI
Molecular formula:	$C_{15}H_{24}O$ to $C_{21}H_{36}O$
Molecular weight range:	Variable: 220 (C9 alkyl derivative) 234 (C10 alkyl derivative) 248 (C11 alkyl derivative) 262 (C12 alkyl derivative) 276 (C13 alkyl derivative) 290 (C14 alkyl derivative) 304 (C15 alkyl derivative)

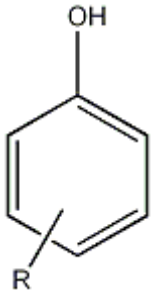
It is important to note that the alternative CAS numbers and names shown below also describe the same substance. Phenol, (tetrapropenyl) derivatives. (CAS 74499-35-7) is the most appropriate CAS name and number available, and the name tetrapropenylphenol (TPP) is most often used to describe this substance. However CAS 74499-35-7 has not been listed on the EINECS inventory, therefore most manufacturers and importers have used the phenol, dodecyl-, branched descriptor with CAS 121158-58-5 (or other dodecyl descriptors listed below) and related EINECS numbers to describe their substances which in fact are derived from a tetrapropene feedstock where C_{12} usually predominates.

A more meaningful name to describe this UVCB substance would perhaps be: phenol, alkylation products with C_{10}-C_{15} branched olefins derived from propene oligomerisation.

Alternative identifiers:

CAS 74499-35-5 Phenol, (tetrapropenyl) derivatives
CAS 27193-86-8 Phenol, dodecyl
CAS 104-43-8 Phenol, 4-dodecyl

Structural formula:



Where R =branched C9, C10, C11, C12, C13, C14 or C15 branched alkyl chain: nominally referred to as tetrapropenyl-derivatives. See composition details, below.

1.2 Composition of the substance

Table 6: Constituents

Constituent	Typical concentration	Concentration range	Remarks
Phenol, alkyl branched (species comprising nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl constituents) CAS 121158-58-5 EC 310-154-3	100%	-	-
Phenol, nonyl-, branched CAS 90481-04-2 EC 291-844-0	0.6% (w/w)	≥0% - ≤4.7%	Typical concentration: average value 2009
Phenol, decyl-, branched	5.3% (w/w)	≥0.5% - ≤20%	Typical concentration: average value 2009
Phenol, undecyl-, branched	16.5% (w/w)	≥12.7% - ≤35.4%	Typical concentration: average value 2009
Phenol, dodecyl-, branched	70.3% (w/w)	≥57.0% - ≤76.9%	Typical concentration: average value 2009 'Phenol, dodecyl-, branched' is assigned CAS 121158-58-5 and EC 310-154-3, the same as the UVCB substance and is therefore not also used to describe a single constituent
Phenol, tridecyl-, branched	4.6% (w/w)	≥1.7% - ≤11.4%	Typical concentration: average value 2009
Phenol, tetradecyl-, branched	1.3% (w/w)	≥0.4% - ≤4.1%	Typical concentration: average value 2009
Phenol, pentadecyl-, branched	1.4% (w/w)	≥0.1% - ≤2.8%	Typical concentration: average value 2009

Current Annex VI entry: Not applicable

Table 7: Impurities

Impurity	Typical concentration	Concentration range	Remarks
-	-	-	None

Current Annex VI entry: Not applicable

Table 8: Additives

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

Current Annex VI entry: Not applicable

1.3 Physicochemical properties

Table 9: Summary of physicochemical 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 Tremain & Atwal, 2010 [test material tetrapropenyl phenol 97.5% purity) Morris, 1997	Observation based on three reports
Melting/freezing point	-9 °C at 1013 hPa	Chevron (2005): test material tetrapropenyl phenol	Pour point method, ASTM D 5950. Measuring a pour point was considered more appropriate than a melting point
Boiling point	In a thermogravimetric Analysis test (TGA, Chevron ILT Test 10301), the TGA analysis indicated: 5% loss @ 189 °C 50% loss @ 246 °C 95% loss @ 270 °C	(Seary 2005) test material tetrapropenyl phenol	A boiling range was reported. A commercial (>99% purity) sample analysed by a thermogravimetric analysis method gave the preferred value of 189-270 °C (Seary 2005).
Relative density	0.9415 at 20 °C	Oronite (1993)	-
Vapour pressure	0.011 Pa at 25 °C	Tremain & Atwal, 2010 test material tetrapropenyl phenol, 97.5% purity	-
Surface tension	42.2 mN/m (90% saturated solution) at 22.0 ± 0.5°C,	Woolley & O'Connor, 2010 test material tetrapropenyl phenol	The test item is considered to be a surface-active item
Water solubility	1.54 mg/L at 20 °C	Mullee (2004).	-
Partition coefficient n-octanol/water	Log Kow (Pow): 7.14 at 25 °C	Dutta 2003: test material tetrapropenyl phenol	Determined using slow stirring method, assumed to be at 25°C. The modeled value based on KOWWIN v1.67 was 7.17 for Log Kow
Flash point	162 °C at 1013 hPa	Woolley & O'Connor, 2010 Oronite Additives, 2003 test material tetrapropenyl phenol Chevron Oronite Company, LLC 1993	-
Flammability	Not applicable	NA	Study not technically feasible
Explosive properties	Not applicable	NA	Based on the known chemical and physical properties a negative result is predicted
Self-ignition temperature	384 °C at 1013 hPa	Woolley &	-

Property	Value	Reference	Comment (e.g. measured or estimated)
		O'Connor, 2010 test material tetrapropenyl phenol	
Oxidising properties	Not applicable	NA	Based on the known chemical and physical properties a negative result is predicted
Granulometry	Not applicable	NA	Registered substance is a liquid
Stability in organic solvents and identity of relevant degradation products	Not applicable	NA	-
Dissociation constant	Not required	CompuDrug, 2010	The dissociation constant (pKa) of a representative structure of Phenol, (tetrapropenyl), derivative (C12 model compound) was estimated using the pKalc function of the PALLAS estimation software program (CompuDrug, version 3.6.2.2, 2006). The pKa was determined to be 9.87. The dissociation constant study does not need to be conducted as the substance is a highly variable complex mixture and therefore the analytical method is unlikely to be sufficiently sensitive. The model value justifies the waiver argument presented in the CSR.
Viscosity	450 cSt at 40 °C and 9 cSt at 100 °C	Oronite, 1993	-

2 MANUFACTURE AND USES

2.1 Manufacture

IU number	Identified Use (IU) name	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 monomer used in chemical synthesis processes and as such it is not a requirement to specify the method of manufacture for the purposes of this CLH proposal.

Phenol, dodecyl-, branched is manufactured in a continuous plant. Phenol and an olefin are reacted together over an ion exchange resin catalyst, to produce phenol, dodecyl-branched. The reaction is exothermic. This occurs over a two reactor system linked in series. The product, exiting the reactors, is passed through a distillation column where any impurities are removed; ensuring the final phenol, dodecyl-, branched product is to the required specification.

2.2 Identified uses

Chemical industry-used in synthesis of polymers from monomers

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

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physicochemical studies

Method	Results	Remarks	Reference
Flash point	162 °C at 1013 hPa	Woolley & O'Connor, 2010 Oronite Additives, 2003 Chevron Oronite Company, LLC 1993	-
Flammability	Not applicable	NA	Study not technically feasible
Explosive properties	Not applicable	NA	Based on the known chemical and physical properties a negative result is predicted
Self-ignition temperature	384 °C at 1013 hPa	Woolley & O'Connor, 2010	-

3.1 Physicochemical properties

3.1.1 Summary and discussion of physic-chemical properties

None of the reported physicochemical properties of phenol, dodecyl-, branched result in a requirement for classification using the criteria set out in the CLP Regulation or in the Dangerous Substances Directive

3.1.2 Comparison with criteria

Phenol, dodecyl-, branched does not meet the criteria for classification on the basis of its physichochemical properties according to the criteria of the CLP Regulation or the Dangerous Substances Directive.

3.1.3 Conclusions on classification and labelling

Phenol, dodecyl-, branched is not classified on basis of physical chemical properties in accordance with CLP criteria.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

No toxicokinetics studies were available to directly address absorption, distribution, metabolism, or excretion of phenol, dodecyl-, branched (tetrapropenyl phenol; TPP). Information from existing toxicology studies was used to infer potential toxicokinetic properties. The systemic availability of phenol, dodecyl-, branched (tetrapropenyl phenol) is dependent the degree to which it is absorbed across body surfaces. Factors that affect absorption include water solubility, lipophilicity (characterized by log Kow), degree of ionisation (the dissociation constant, pKa) and molecular size. The physical state of the compound is an oily liquid at 20ºC and 101.3 KPa. The compound is lipophilic, with an estimated log Kow of 7.14 and the estimated water solubility is 1.54 mg/L for the main component, which is considered slightly soluble (0.1-100 mg/L). The substance is expected to be present in its non-ionised form at environmentally relevant pH.

4.1.1 Non-human information

Oral exposure

Based on its high lipophilicity and low water solubility, phenol, dodecyl-, branched (tetrapropenyl phenol; TPP) is expected to be absorbed into and through the cell membrane and to subsequently have a wide systemic distribution. Effects seen in repeated dose (sub-acute and sub-chronic) toxicity studies confirm that phenol, dodecyl-, branched (tetrapropenyl phenol) is distributed throughout the body after oral administration. In a 28-day repeated dose oral toxicity study (Harriman, 2004), effects were apparent in the liver and reproductive organs of both male and female rats. A NOAEL was established at 60 mg/kg bw/d based on organ weight effects and microscopic findings in the liver at 60 mg/kg bw/d and higher that disappear after recovery, indicating elimination from the tissue. However, effects in other tissues continued to be seen after the recovery period at higher doses which could be due to metabolic overload.

Organ weight and histopathological effects were noted in two 90-day study in rats (Vogin, 1970; Haas, 2007). The results of these studies indicate that phenol, dodecyl-, branched is systemically absorbed. Whole body distribution is also supported by the results of reproductive toxicity studies.

Dermal exposure

Phenol, dodecyl-, branched (tetrapropenyl phenol) is predicted to penetrate the dermis and circulate throughout the body based on its moderate molecular weight by formula (<500 g/mole) and its high lipophilicity. This prediction is supported by data in rabbits (Randall & Robinson, 1978) showing that at high acute doses, clinical signs included gross effects on the lung, liver, spleen, kidney, gall bladder, and the gastrointestinal tract. These organs were histologically normal by the end of the observation period, indicating that the compound was eliminated from the target tissues over time.

Inhalation Exposure

Phenol, dodecyl-, branched only exists in liquid form and under normal conditions of use will not be found as an aerosol. Information relating to its vapour pressure suggests the substance is unlikely to be inhaled. Exposures via inhalation leading to absorption through the respiratory system are therefore unlikely but on the basis of its physicochemical properties, it may be expected to penetrate biological membranes and be systemically available.

4.1.2 Human information

No data are available.

4.1.3 Summary and discussion on toxicokinetics

In summary, the absorption, distribution, metabolism, and excretion of phenol, dodecyl-, branched (tetrapropenyl phenol) has not been directly studied *in vivo*. However, toxicology studies show that tetrapropenyl phenol crosses biological membranes, resulting in systemic distribution. While the compound appears to be eliminated from tissues, based on recovery from tissue effects seen in a sub-acute oral study (Harriman 2004) and in an acute dermal study (Randall & 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.

4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
ORAL EXPOSURE			
Acute rat oral toxicity: equivalent or similar to OECD Guideline 401 (Acute Oral Toxicity)	LD50: 2100 mg/kg bw (male/female) based on: test material.	Rat LD50 oral Test material: Phenol, (tetrapropenyl) derivatives (CAS 27193-86-8) ~93% p-dodecylphenol, ~7% o-dodecylphenol	Randall D & Robinson E (1978)
Acute oral toxicity study in male and female rats.	LD50: 2200 mg/kg bw (male/female) based on test material (EC name) Phenol, dodecyl-, branched	Rat LD50 oral Test material: CAS 11067-80-4 (97% purity)	Mürmann P (1984a) OECD SIDS(2006)
Acute rat oral toxicity: method similar to FHSA 16CFR1500.3	LD50: 500-5000 mg/kg bw (male) based on test material (EC name): Phenol, dodecyl-, branched	Rat LD50 oral Test material: Phenol, (tetrapropenyl) derivatives (CAS 74499-35-7	Cavalli, Hallesy & Spence (1968)
Acute rat oral toxicity equivalent or similar to 40 CFR 772.112-21	LD50: <5000 mg/kg bw (male/female) based on test material (EC name) Phenol, dodecyl-, branched	Rat LD50 oral Test material: Phenol, (tetrapropenyl) derivatives (CAS 74499-35-7	Costello & Gilman (1982)
DERMAL EXPOSURE			
Acute rabbit dermal toxicity equivalent or similar to OECD Guideline 402 coverage-occlusive	LD50: ca. 15000 mg/kg bw (male) based on test material (EC name): Phenol, dodecyl-, branched	Rabbit LD50 dermal: ca. 15 g/kg bw Test material: Phenol, (tetrapropenyl) derivatives (CAS 74499-35-7	Cavalli, Hallesy & Spence (1968)
Acute rabbit dermal toxicity Coverage: semi-occlusive	LD50: >2000 mg/kg bw (male/female) based on: test material (EC name): Phenol, dodecyl-, branched	Rabbit LD50 dermal: >2000 mg/kg bw Test material: Phenol, (tetrapropenyl) derivatives (CAS 27193-86-8) ~93% p-dodecylphenol, ~7% o-dodecylphenol	Randall D & Robinson E (1978)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

In the acute oral toxicity studies the median lethal dose was found to exceed the relevant limit dose of 2000 mg/kg bw. No classification for acute oral toxicity is warranted on the basis of these results.

4.2.1.2 Acute toxicity: inhalation

Investigation of acute inhalation toxicity were not performed and are not considered appropriate on the basis that the vapour pressure of the substance is very low (<0.1 Pa at 20°C), phenol, dodecyl-, branched only exists in liquid form and it will not be aerosolised in its normal pattern of use. No classification for acute inhalation toxicity is required.

4.2.1.3 Acute toxicity: dermal

Both of the acute dermal toxicity studies in rabbits, using occluded or semi-occluded application methods, showed no mortality following a single topical application of phenol, dodecyl-, branched at dose levels of 2000 mg/kg bw or higher. No classification for acute dermal toxicity is therefore warranted on the basis of these results.

4.2.1.4 Acute toxicity: other routes

Not applicable: no available data.

4.2.2 Human information

No acute toxicity data are available.

4.2.3 Summary and discussion of acute toxicity

Phenol, dodecyl-, branched was found to be of low acute toxicity following oral and dermal administration. Investigations of acute inhalation toxicity are not required due to the physicochemical properties of the substance and its pattern of use under normal conditions. There were no findings in any of the acute toxicity studies to indicate adverse effects of single phenol, dodecyl-, branched exposure to rats or rabbits.

4.2.4 Comparison with criteria

Phenol, dodecyl-, branched does not meet any of the classification criteria set out in the CLP Regulation (EC) No. 1272/2008 (Annex I: 3.1.2.1, Table 3.1.1. The results of the various acute toxicity studies were greater than the upper levels of the Category 4 range for oral and dermal exposure. No classification is required for acute inhalation toxicity in the absence of any data

4.2.5 Conclusions on classification and labelling

No classification is warranted for acute exposure by oral, dermal routes or inhalation routes of exposure according to the criteria specified in the CLP Regulation. No classification for acute toxicity is required according to criteria specified in the Dangerous Substances Directive.

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

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

No findings indicating any concerns of relevance to specific target organ toxicity (single exposure) (STOT-SE) were observed following administration by oral or dermal routes.

4.3.2 Comparison with criteria

The guidance values set out in Table 3.8.2 of Guidance on the Application of CLP Criteria, Point 3.8.2.2.1 for oral, dermal and inhalation exposure routes do not indicate that classification with STOT-SE is required for phenol, dodecyl-, branched. There were no effects with a potential to cause adverse reaction or be potentially harmful to humans and no transient respiratory tract irritation that would require classification of the substance in Cat 3 STOT-SE.

4.3.3 Conclusions on classification and labelling

No classification is required with regard to acute oral, dermal or inhalation toxicity.

4.4 Irritation

4.4.1 Skin irritation

Table 13: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Method used was equivalent to or similar to OECD Guideline 404 (Acute Dermal Irritation / Corrosion) Coverage: semi-occlusive (NZW rabbits were shaved and had 2 intact and 2 abraded sites)	Irritating Primary dermal irritation index (PDII): 6.2 Test material (EC name): Phenol, dodecyl-, branched	For intact skin: Erythema score: 4 of max. 4 (mean) (Time point: 24 h) (not fully reversible within: 14 days) 4 of max. 4 (mean) (Time point: 48 h) (not fully reversible within: 14 days) 4 of max. 4 (mean) (Time point: 72 h) (not fully reversible within: 14 days) Oedema score: 3.4 of max. 4 (mean) (Time point: 24 h) (fully reversible within: 7 days) 2.6 of max. 4 (mean) (Time point: 48 h) (fully reversible within: 7 days) 2.8 of max. 4 (mean) (Time point: 72 h) (fully reversible within: 7 days). Further details on the test material held by the sponsor.	Waid, Dougherty & Wong (1989)
Method-Federal Hazardous Substances Act, 21 CFR, § 191.11 (1964). Coverage: occlusive (rabbits were shaved and had intact and abraded sites)	Irritating Primary dermal irritation index (PDII): 6 Test material (EC name): Phenol, dodecyl-, branched	For intact skin: Erythema score: 2.3 of max. 3 (mean) (Time point: 24 h) (not fully reversible within: 72 h) 4 of max. 4 (mean) (Time point: 72 h) (not fully reversible within: 72 h) Oedema score: 2.8 of max. 4 (mean) (Time point: 24 h) (not fully reversible within: 72 h) 3 of max. 3 (mean) (Time point: 72 h) (not fully reversible	Cavalli, Hallesy & Spence (1968)

Method	Results	Remarks	Reference
		within: 72 h) Test material: details not specified	
Method-Federal Hazardous Substances Act, 21 CFR, § 191.11 (1964). Coverage: semi-occlusive (NZW rabbits were shaved)	Highly irritating Primary dermal irritation index (PDII): 8	Test material: Phenol, (tetrapropenyl) derivatives (CAS 27193-86-8) ~93% p-dodecylphenol, ~7% o-dodecylphenol	Randall D & Robinson E (1978)
Rabbit	Irritating Primary dermal irritation index (PDII): 6.09 Test material (EC name): Phenol, dodecyl-, branched	Supporting study only Erythema score: 4 (mean) (Time point: 24 h) (not fully reversible within: 72 h) 4 (mean) (Time point: 48 h) (not fully reversible within: 72 h) 4 (mean) (Time point: 72 h) (not fully reversible within: 72 h) Oedema score: 2.7 (mean) (Time point: 24 h) (not fully reversible within: 72 h) 2 (mean) (Time point: 48 h) (not fully reversible within: 72 h) 2 (mean) (Time point: 72 h) (not fully reversible within: 72 h) Test material: details not specified	Mürmann, P (1984b) OECD SIDS(2006)
Rabbit	Irritating Primary dermal irritation index (PDII): 6.75 Test material (EC name): Phenol, dodecyl-, branched	Supporting study only Erythema score: 4 (mean) (Time point: 24, 48 & 72 h) (not fully reversible within: 72 h) Oedema score: 4 (mean) (Time point: 24, 48 & 72 h) (not fully reversible within: 72 h) Test material: details	Mürmann, P (1988) OECD SIDS(2006)

Method	Results	Remarks	Reference
		not specified	
Rabbit	Irritating Primary dermal irritation index (PDII): 6.5 Test material (EC name): Phenol, dodecyl-, branched	Supporting study only Erythema score: 4 (mean) (Time point: 24, 48 & 72 h) (fully reversible within: 17 days) (Healed with scar formation) Oedema score: 4 (mean) (Time point: 24, 48 & 72 h) (fully reversible within: 17 days) (Healed with scar formation) Test material: details not specified	Mürmann, P (1991) OECD SIDS(2006)

4.4.1.1 Non-human information

In the key, guideline compliant, standard three rabbit test, some degree of erythema and oedema was observed for all animals at each observation time, with recovery generally not complete within 72 hours. A number of supporting studies are presented-all showing a similar pattern of response with an irritant response indicated following single topical application for at least 4 hours.

4.4.1.2 Human information

No data are available.

4.4.1.3 Summary and discussion of skin irritation

In the standard three rabbit test, a degree of erythema and/or oedema was observed at all application sites of the animals at each observation time, generally not resolving within 72 hours of application and requiring classification of phenol, dodecyl-, branched as a skin irritant according to the criteria defined in the CLP Regulation. Classification as a skin irritant (R36: irritating to skin) is required according to the criteria of the Dangerous Substances Directive

4.4.1.4 Comparison with criteria

Conclusions on classification and labelling

Classification for skin irritation is required according to the criteria specified in Regulation (EC) No. 1272/2008. Phenol, dodecyl-, branched is classified for skin irritation (Category 2): H315-Causes skin irritation. Classification as a skin irritant (R36: irritating to skin) is required according to the criteria of the Dangerous Substances Directive

4.4.2 Eye irritation

Table 14: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Method equivalent or similar to OECD Guideline 405 (Acute Eye Irritation / Corrosion) in NZW rabbits Test material (EC name): Phenol, dodecyl-, branched	Not irritating	Cornea score: 0 (mean) (Time point: 24, 48 & 72 h) (no effects seen) Iris score: 0 (mean) (Time point: 24, 48 & 72 h) (no effects seen) Conjunctivae score: 1.8 of max. 3 (mean) (Time point: 24 h) (fully reversible within: 10 days) 1.7 of max. 3 (mean) (Time point: 48 h) (fully reversible within: 10 days) 1.3 of max. 2 (mean) (Time point: 72 h) (fully reversible within: 10 days) Chemosis score: 0.3 of max. 1 (mean) (Time point: 24 h) (fully reversible within: 48 h) Test material: details not specified	Waid, Rogers & Wilkenfeld (1990)
Method equivalent or similar to OECD Guideline 405 (Acute Eye Irritation / Corrosion) in NZW rabbits Test material (EC name): Phenol, dodecyl-, branched	Irritating	Cornea score: 1.7 of max. 4 (mean) (Time point: 24 h) (not fully reversible within: 72 h) 0.7 of max. 3 (mean) (Time point: 48 h) (not fully reversible within: 72 h) 1 of max. 4 (mean) (Time point: 72 h) (not fully reversible within: 72 h) Iris score: 0.5 of max. 1 (mean) (Time point: 24, 48 & 72 h) (not fully reversible within: 72 h) Conjunctivae score: 3 of max. 3 (mean) (Time point: 24 h) (not fully reversible	Cavalli, Hallesy & Spence (1968)

Method	Results	Remarks	Reference
		within: 72 h) 2.7 of max. 3 (mean) (Time point: 48 h) (not fully reversible within: 72 h) 2.3 of max. 3 (mean) (Time point: 72 h) (not fully reversible within: 72 h) Chemosis score: 3.3 of max. 4 (mean) (Time point: 24 h) (not fully reversible within: 72 h) 3.5 of max. 4 (mean) (Time point: 48 h) (not fully reversible within: 72 h) 3 of max. 4 (mean) (Time point: 72 h) (not fully reversible within: 72 h) Test material: details not specified	
Method-Federal Hazardous Substances Act, 21 CFR, § 191.12 (1964) using NZW rabbits. Test material (EC name): Phenol, dodecyl-, branched	Moderately irritating	Mean overall irritation score: 33.3 No timepoint information Test material: Phenol, (tetrapropenyl) derivatives (CAS 27193-86-8) ~93% p-dodecylphenol, ~7% o-dodecylphenol	Randall D & Robinson E (1978)
Rabbit 0.1 mL of the test material (97% purity) was applied to the eyes of six rabbits. They were observed for up to 21 days. Test material (EC name): Phenol, dodecyl-, branched	Mean overall irritation score: 19.3 (Time point: 24 hours)	Mean overall irritation score: 19.3 (Time point: 24 h) Mean conjunctival score: 2.5 (Time point: 24 h) 2.2 (Time point: 48 h) 2 (Time point: 72 h) No data on reversibility. Test material: details not specified	Anon (2006) Mürmann P (1984c)

4.4.2.1 Non-human information

In a number of key or supporting ocular irritation tests, the treated rabbit eyes developed conjunctival reactions that generally did not resolve within 72 hours of instillation and, in some cases, persisted for up to 10 days after exposure. Instillation of the test material resulted in ocular

irritation, which was predominantly evident as conjunctival redness. Relevant classification thresholds were exceeded on the basis of these results.

4.4.2.2 Human information

No data are available.

4.4.2.3 Summary and discussion of eye irritation

Irritant reactions were evident in treated rabbit eyes assessed over 72 hours following instillation. Typical responses included predominantly conjunctival reactions, with mean scores that exceeded the classification thresholds. No human data are available.

4.4.2.4 Comparison with criteria

The ocular responses observed in various studies require classification of the substance for serious eye irritation (Category 2) H319-Causes serious eye irritation under CLP. Classification as an eye irritant (R38: irritating to eyes) is required according to the criteria of the Dangerous Substances Directive

4.4.2.5 Conclusions on classification and labelling

The ocular responses observed in various studies require classification of the substance for serious eye irritation (Category 2) H319-Causes serious eye irritation under CLP. Classification as an eye irritant (R38: irritating to eyes) is required according to the criteria of the Dangerous Substances Directive

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

No data are available. The acute inhalation toxicity of the substance has not been investigated: the physicochemical properties and use pattern of the substance indicate that significant inhalation exposure will not occur.

4.4.3.2 Human information

No data are available.

4.4.3.3 Summary and discussion of respiratory tract irritation

There are no indications from available information that phenol, dodecyl-, branched is likely to result in respiratory tract irritation. Due to low volatilisation potential and the normal use pattern, the possibility of inhalation exposure is considered to be low.

4.4.3.4 Comparison with criteria

In the absence of any evidence of respiratory irritation from animal studies or from human exposure, classification as a respiratory irritant is not required according to CLP or DSD.

4.4.3.5 Conclusions on classification and labelling

No classification is indicated in the absence of any evidence of respiratory tract irritation in humans or experimental animals.

4.5 Corrosivity

There were no indications of a corrosive response in any of the reported acute toxicity or irritation studies, detailed above. Phenol, dodecyl-, branched is not corrosive in contact with skin, mucus membranes or eyes and is not expected to be corrosive under single or repeated exposure scenarios.

Table 15: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
No study available	NA	NA	NA

4.5.1 Non-human information

Skin and eye irritation studies are discussed in Section 4.4.2, above. No evidence of a corrosive response was observed in any of these studies. No additional studies specifically addressing corrosivity are available.

4.5.2 Human information

No data are available.

4.5.3 Summary and discussion of corrosivity

No evidence of corrosivity was observed in studies investigating the skin and eye irritation of phenol, dodecyl-, branched.

4.5.4 Comparison with criteria

No evidence of corrosion was observed in the skin irritation studies reported in Table 13.

4.5.5 Conclusions on classification and labelling

Phenol, dodecyl-, branched does not require classification for corrosive properties according to CLP or DSD criteria.

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

within 72 h.

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

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

Irritation parameter	**Time point**	**Mean score in 6 animals**	**Remarks**
Erythema score	24 h	4	Eschar (in 6 rabbits)
	48 h	4	Eschar (in 6 rabbits)
	72 h	4	Eschar (in 6 rabbits), cracking (in 1 rabbit)
	7 days	4	Eschar, cracking, thin layer of light brown necrotic skin, thick light brown flakes-flaky (in all rabbits).
	14 days	**2.8**	Thin layer of light brown necrotic skin (in 1 rabbit), thick light brown flakes/flaky (in all rabbits), alopecia (in 3 rabbits), thin fur regrowth (in 4 rabbits), sloughing (in 1 rabbit).
Oedema score	24 h	3.4	Fully reversible within 7 days.
	48 h	2.6	
	72 h	2.8	
	7 days	0	
	14 days	0	

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

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

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

2) In Cavalli *et al.* (1968), a dermal irritation study (supporting study; according to guideline: Federal hazardous substances act methods, 21 CFR; not GLP; Klimisch score:

2) six rabbits received a single 24-h (instead of 4-h) application of 0.5 ml TPP to intact and abraded skin. Irritation was scored at 24 and 72 h after removal of the test material and at 7 days, using the Draize scoring method.

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

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

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

3) Another supporting study, Randall & Robinson, 1990, using six rabbits is also available for this endpoint. The study is considered to have a reliability Klimisch score of 2 as there is limited information available. The report is limited to a summary of the study and there is no individual animal data. The dermal exposure lasted for 24 h instead of 4 h as required in OECD TG 404. The test material used, Phenol, (tetrapropenyl) derivatives, has been assigned another CAS number (CAS 27193-86-8) than the one used by the DS (121158-58-5). The purity was given as: ~93% p-dodecylphenol, contaminated with ~7% o-dodecylphenol. The resulting Primary irritation score was 8.0/8.0.

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

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

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

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

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

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

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

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

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

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

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

(day 17; Mürmann, 1991)

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

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

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

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

1. In the Waid *et al*. (1990) eye irritation study (comparable to OECD TG 405; according to GLP; reliability - Klimisch score: 1), 0.1 ml of the test material was placed in the conjunctival sac of one eye in each of 9 rabbits. Three of the rabbits were further treated after a 30-second exposure by rinsing the eyes for 1 minute with distilled water at a rate of approximately 250 millilitres per minute. Eye irritation was scored at 1, 24, 48 and 72 h after exposure and at 7 and 14 days, using the Draize scoring method. According to the conclusion provided in the summary of the study results, rinsing of eyes did not significantly affect the severity or persistence of irritation. Therefore, in the table below only effects in rabbits with un-rinsed eyes are presented.

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

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

	48 h	0	-	
	72 h	0	-	

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

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

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

Irritation parameter	**Time point**	**Mean score of 6 rabbits**	**Mean for 24, 48, and 72 h**	**Reversibility**
Cornea opacity score	24 h	1.7	1.1 (effect only in 3 out of 6 rabbits)	Not fully reversible within 72 h.
	48 h	0.7		
	72 h	1		
Iris lesion score	24 h	0.5	0.5 (effect in 3 of 6 rabbits)	
	48 h	0.5		
	72 h	0.5		
Conjunctivae redness score	24 h	3	**2.7** (effect in 6 of 6 rabbits)	
	48 h	2.7		
	72 h	2.3		
Chemosis score	24 h	3.3	**3.3** (effect in 6 of 6 rabbits)	
	48 h	3.5		
	72 h	3		

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

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

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

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

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

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

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

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

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

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

Taking into account the poor reporting and inconsistencies in results from the available studies, a weight of evidence approach was used, taking the Cavalli *et al*. study (1968), in which the effects were most pronounced, as the most informative study and the other studies as supportive. Unfortunately, the reversibility of effects after 72 h after eye instillation was not followed in the Cavalli *et al.* study; thus reversibility is assessed based on all available studies.

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

4 out of 6 rabbits show:

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

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

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

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

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

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

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

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

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

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

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

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

4.6 Sensitisation

4.6.1 Skin sensitisation

The results from two skin sensitisation tests, performed according to the Buehler method, did not indicate any potential of the substance to cause skin sensitisation (delayed contact hypersensitivity).

Table 16: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Buehler test, OECD Guideline 406 (Skin Sensitisation) Induction: epicutaneous, occlusive Challenge: epicutaneous, occlusive Hartley guinea pig male/female	Not sensitising No. with positive reactions: 1st reading: 1 out of 10 (negative control); 24 h after challenge.; dose: 1% w/v in mineral oil 1st reading: 1 out of 19 (test group); 24 h after challenge; dose: 1% w/v in mineral oil Re-challenge: 3 out of 20 (test group); 24 h after challenge.; dose: 1% w/v in mineral oil 2nd reading: 0 out of 10 (negative control); 48 h after challenge.; dose: 1% w/v in mineral oil 2nd reading: 1 out of 19 (test group); 48 h after challenge; dose: 1% w/v in mineral oil Re-challenge: 3 out of 20 (test group); 48 h after challenge; dose: 1% w/v in mineral oil	Test material (EC name): Phenol, dodecyl-, branched: details not specified	Morris (1997)
Buehler test, OECD Guideline 406 (Skin Sensitisation) Induction: epicutaneous, occlusive Challenge: epicutaneous, occlusive Hartley guinea pig male/female	Not sensitising No. with positive reactions: 1st reading: 0 out of 10 (negative control); 24 h after challenge; dose: mineral oil 1st reading: 0 out of 15 (test group); 24 h after challenge; dose: 5% w/v in mineral oil 2nd reading: 0 out of 10 (negative control); 48 h after challenge; dose: mineral oil 2nd reading: 3 out of 15 (test group); 48 h after challenge; dose: 5% w/v in mineral oil	Test material (EC name): Phenol, dodecyl-, branched: details not specified	Silveira *et al* (1983)

4.6.1.1 Non-human information

In two studies (performed according to the Buehler method) in Guinea Pigs, phenol, dodecyl-, branched was administered by occluded epicutaneous application during the induction phase and subsequently used for challenged (1-5% concentration in mineral oil). None of the responses exceeded the threshold for classification as a skin sensitiser and in most cases there were no signs of a dermal response in guinea pigs. No reactions indicative of contact hypersensitivity were noted.

4.6.1.2 Human information

No data are available.

4.6.1.3 Summary and discussion of skin sensitisation

The key study for skin sensitisation (Morris, 1997) was conducted according to OECD 406 and GLP. Some background irritation was observed among the naive control animals. This study was conducted most recently and was considered to be the most reliable study. No reactions indicative of delayed contact hypersensitivity were observed. In the supporting study for skin sensitisation the methodology suggests that it was conducted similarly to OECD 406. The study was conducted in accordance with GLP. No sensitisation reactions were observed in the 15 animals induced and challenged with the test material.

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

4.6.1.4 Comparison with criteria

Classification for skin sensitisation is not required according to CLP or DSD criteria.

4.6.1.5 Conclusions on classification and labelling

There is no evidence for sensitisation in two Guinea Pig studies performed according to the method of Buehler. No human data are available. Phenol, dodecyl-, branched does not therefore require classification for skin sensitisation according to Regulation (EC) No. 1272/2008 or the Dangeous Substances Directive.

4.6.2 Respiratory sensitisation

No data are available.

Table 17: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference
No study available	NA	No information	NA

4.6.2.1 Non-human information

No data are available.

4.6.2.2 Human information

No data are available.

4.6.2.3 Summary and discussion of respiratory sensitisation

No data are available on which to base an assessment of hazard.

4.6.2.4 Comparison with criteria

(a) .
No data are available to assess the respiratory sensitisation potential of phenol, dodecyl-, branched. Phenol, dodecyl-, branched does not therefore require classification for respiratory sensitisation according to Regulation (EC) No. 1272/2008 or the Dangeous Substances Directive.

4.6.2.5 Conclusions on classification and labelling

No classification indicated for respiratory sensitisation.

4.7 Repeated dose toxicity

Table 18: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Rat (Crl:CD(SD)IGS BR) male/female subacute (oral: gavage) 0, 30, 75, 150, 300, 500 mg/kg bw/d (actual ingested) Exposure: 14 days (Daily gavage) equivalent or similar to OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)	NOAEL: 75 mg/kg bw/day (actual dose received) (male/female) based on: test mat. (Clinical signs and organ weight effects noted at 150 mg/kg bw/day) LOAEL: 150 mg/kg bw/day (actual dose received) (male/female) based on: test mat. (Clinical signs and organ weight effects)	Treated animals showed a spectrum of systemic toxicity including a) mortality at 500 mg/kg bw/d, b) clinical observations of perianal staining, hair loss, soft faeces, clear yellow/brown material on body surfaces and unkempt appearance at doses ≥150 mg/kg bw/d, c) body weight (absolute and gain) reductions among males at doses ≥ 300 mg/kg bw/d and d) an increase in adrenal weights (suggestive of a stress response) at doses ≥ 300 mg/kg bw/day. Gross necropsy revealed small prostate and seminal vesicles in the two surviving 500 mg/kg bw/d males; small seminal vesicles were also observed in one male at 300 mg/kg bw/d. Reduced absolute and relative weights of the prostate, seminal vesicles and epididymides were observed in males at ≥150 mg/kg bw/d. Absolute testes weights were lower in these dose groups however relative weights were comparable to controls, indicating that these effects were secondary to reduced bodyweight. No treatment-related findings were reported for the female reproductive tract in this study. Test material: tetrapropenyl phenol (CAS 74499-35-7), 100% purity	Harriman, J.F. (2004)
Sub-acute 28 day (7 days/week) oral exposure by gavage at dose	NOAEL: 60 mg/kg bw/d (male/female) based on: test material-(EC name): Phenol,	Organ weight and/or microscopic findings in the reproductive organs	Harriman, JF (2004)

levels of : 0, 5, 20, 60, 180, 300 mg/kg bw/d OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents) Rat (Crl:CD®(SD)IGS BR) male/female	dodecyl-, branched. NOEL: <5 mg/kg bw/d (male rats) based on actual dose of test material received. NOEL: 20 mg/kg bw/d (female rats) based on actual dose of test material received	persisted to the recovery necropsy at 60 mg/kg bw/d. Microscopic finding of follicular cell hypertrophy in the thyroid glands in one male dosed at 5 mg/kg bw/d at the primary necropsy. Clinical observations noted at 60 mg/kg bw/d in females Test material: tetrapropenyl phenol (CAS 74499-35-7), 100% purity	
Sub-acute oral exposure via dietary inclusion at 0, 500, 2500 and 5000 ppm nominal in the diet Exposure: Four week treatment duration (7 days/week) equivalent or similar to OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents) rat (Sprague-Dawley) male/female.	NOEL: 500 ppm (male/female) based on test material-Phenol, dodecyl-, branched	Reduced food consumption, weight loss or reduced weight gain was seen at the highest dose level, effects on both food consumption and weight gain also apparent at 2500 ppm. Red-coloured urine was observed in both sexes at 5000 ppm and in females at 2500 ppm. Adrenal weights were increased in males at the highest dose level; liver weights were increased in females in this group. Gross necropsy revealed treatment-related changes in the reproductive tract (small/atrophic prostate and seminal vesicles, small and/or soft testes) in eight males at the highest dose level. Findings were confirmed histopathologically in seven of these rats and consisted of epididymal hypoplasia and hypospermia, reduced prostatic secretion and prostatic hypoplasia, reduced seminal vesicle secretion and seminal vesicle hypoplasia. Test material: phenol (tetrapropenyl) derivates: CAS 27193-86-8	Reyna & Thake (1988)
Sub-chronic oral exposure: 90 day treatment via diet-7	NOEL: 25 mg/kg diet (male/female) based on: test	The results of this study indicate an effect of the	Vogin, E (1970a)

days/week 0, 0.05, 0.2 and 0.4% in the diet (Approximately 25, 100 and 200 mg/kg bw/d) 90 day repeat dose study via feed. Daily observations were recorded. Blood and urine examinations were made in 5 rats per sex per group. The animals were sacrificed after 90 days when gross necropsy and histopathological examinations were performed. rat (Albino rats of the FDRL Strain) male/female	material-Phenol, dodecyl-, branched.	test material on the male reproductive tract at the highest dose level of 200 mg/kg bw/d. Findings in males were associated with reduced weight gain. Test material: Phenol, dodecyl (CAS 27193-86-8)	
rat (Crl:SD(SD)IGS BR) male/female subchronic (oral: feed) 0, 50, 100, 150 and 200 mg/kg bw/day (Dietary concentrations were adjusted weekly, based on predicted bodyweight and food consumption.) Exposure: 91-92 days (Continous - ad libitum dietary exposure) OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	NOAEL (Reproductive tract effects): 100 mg/kg bw/day (nominal) (male/female) based on: test mat. (Mean lower absolute and relative (to bodyweight and brain weight) weights of the epididymides, prostate and seminal vesicles were observed in males at ≥100 mg/kg bw/d.) NOAEL (General toxicity): < 50 mg/kg bw/day (nominal) (male/female) based on: test mat. (A NOAEL for general toxicity cannot be determined for this study however due to bodyweight effects observed at all dose levels.)	No deaths occurred and no clinical signs were observed during the study period although systemic toxicity as evidenced by reductions in absolute body weight and body weight gain, were observed at all treatment levels. Animals dosed at ≥100 mg/kg bw/day also showed reduced food consumption, an effect attributable to toxicity rather than palatability of the diet as rats are known to readily adapt to unpalatable diets and similar reductions in food consumption were observed in other studies where animals dosed by gavage. Other indicators of systemic toxicity included, reductions in red blood cell counts and haemoglobin in males at 200 mg/kg bw/d, and lower white blood cell counts and lymphocyte counts in either sex at 200 mg/kg bw/d, increased mean absolute adrenal weights in males dosed at ≥150 mg/kg bw/d, and increased relative adrenal weights in either sex at this dose level or higher, and among males dosed at ≥100 mg/kg bw/d (suggestive of a stress response). Animals dosed	Haas, M.C. (2011)

		at ≥ 150 mg/kg bw/d also showed periportal hepatocellular vacuolization. Gross necropsy revealed small testes, epididymides, coagulating gland, prostate and seminal vesicles at 200 mg/kg bw/d; small coagulating gland, prostate and seminal vesicles were also observed at 150 mg/kg bw/d. Mean lower absolute and relative (to bodyweight and brain weight) weights of the epididymides, prostate and seminal vesicles were observed in males at ≥100 mg/kg bw/d. Mean lower absolute and higher relative testes weights were observed at 200 mg/kg bw/d; mean relative (to brain weight) testes weights were significantly reduced in males at 200 mg/kg bw/d only. Additionally, mean lower absolute and relative (to bodyweight and brain weight) ovary weights were observed in females at these dose levels. Mean lower testes weight (relative to brain weight) was observed in males at 200 mg/kg bw/d only. Organ weight effects were accompanied by histopathology findings of coagulating gland atrophy and prostate atrophy in males at 200 mg/kg bw/d, decreased secretion in the seminal vesicles in males at ≥150 mg/kg bw/d and decreased corpora lutea in the ovaries in females at ≥150 mg/kg bw/d. There was, therefore, no evidence of an adverse effect of TPP on the reproductive tract that was not accompanied by significant general	

		systemic toxicity, especially reduced bodyweight gain. Test material: tetrapropenyl phenol (CAS 74499-35-7), 100% purity	
Dog (Beagle) male/female Sub-chronic oral exposure via feed at nominally 0, 0.05, 0.2 and 0.4% in the diet. Exposure: 13 week treatment duration; treated feed available 1 hour/day, 6 days/week	NOEL: >200 mg/kg diet (male/female) based on test material-Phenol, dodecyl-, branched	Mean intakes are calculated to be equivalent to approximately 0, 18, 71 and 143 mg/kg bw/d respectively. No deaths occurred and no signs of toxicity were observed during the study period. No treatment-related effects were apparent on organ weights; histopathology did not reveal any effects of treatment. Test material: Phenol, dodecyl (CAS 27193-86-8)	Vogin, E (1970b)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

A 14-day gavage toxicity study in the rat (Harriman, 2003) identified a NOAEL of 75 mg/kg bw/d. Treated animals showed a spectrum of systemic toxicity including a) mortality at 500 mg/kg bw/d, b) clinical observations of perianal staining, hair loss, soft faeces, clear yellow/brown material on body surfaces and unkempt appearance at doses ≥150 mg/kg bw/d, c) body weight (absolute and gain) reductions among males at doses ≥ 300 mg/kg bw/d and d) an increase in adrenal weights (suggestive of a stress response) at doses ≥ 300 mg/kg bw/day. Gross necropsy revealed small prostate and seminal vesicles in the two surviving 500 mg/kg bw/d males; small seminal vesicles were also observed in one male at 300 mg/kg bw/d. Reduced absolute and relative weights of the prostate, seminal vesicles and epididymides were observed in males at ≥150 mg/kg bw/d. Absolute testes weights were lower in these dose groups however relative weights were comparable to controls, indicating that these effects were secondary to reduced bodyweight. No treatment-related findings were reported for the female reproductive tract in this study.

A 28-day dietary toxicity study in the rat (Reyna & Thake, 1988) identified a NOAEL of 180 mg/kg bw/d (2500 ppm). No deaths occurred during the study period, however evidence for systemic toxicity included a) reduced food consumption of 27-49%, weight loss or reduced weight gain at the highest dose level in either sex in this study, with effects on both food consumption and weight gain also apparent at 180 mg/kg bw/d, b) red-coloured urine was observed in either sex at 300 mg/kg bw/d and in females at 180 mg/kg d and c) increased adrenal weights in males (suggestive of a stress response) at 300 mg/kg bw/d. Liver weights were also increased in females in this group. Gross necropsy revealed treatment-related changes in the reproductive tract (small/atrophic prostate and seminal vesicles, small and/or soft testes) in eight males at 300 mg/kg bw/d. Findings were confirmed histopathologically in seven of these rats and consisted of epididymal hypoplasia

and hypospermia, reduced prostatic secretion and prostatic hypoplasia, reduced seminal vesicle secretion and seminal vesicle hypoplasia. Effects of treatment were therefore apparent on the male reproductive tract in this study at 300 mg/kg bw/d. No effects on the male reproductive tract were reported at 180 mg/kg bw/d, a dose level at which less marked systemic toxicity was apparent. No treatment-related findings were reported for the female reproductive tract in this study.

A 28-day gavage toxicity study in the rat (Harriman, 2004), identified a NOAEL of 60 mg/kg bw/d. No deaths occurred during the course of the study. However, general systemic toxicity was evident through a) clinical signs (excessive salivation and perianal staining) at dose levels of ≥60 mg/kg bw/d, b) markedly reduced bodyweights, weight gains and food consumption were seen in males at ≥180 mg/kg bw/d, c) increased adrenal weights (suggestive of a stress response) at ≥180 mg/kg bw/d, d) haematology effects of reduced haemoglobin and haematocrit, reduced lymphocyte counts, and increased reticulocyte counts in females at ≥180 mg/kg bw/d and e) centrilobular hepatocyte hypertrophy and hepatocyte vacuolization in the liver, and hypertrophy of follicular cells of the thyroid in males. At Week 4, gross necropsy revealed small testes, prostate, seminal vesicles, epididymides and/or coagulating gland in males at ≥180 mg/kg bw/d. Mean absolute and relative weights of the seminal vesicles were markedly reduced at 180 and 300 mg/kg bw/d and reduced seminal vesicle secretion was observed in all males at both dose levels. Mean absolute and relative prostate weights were also markedly reduced in these animals together with reduced prostatic secretion. Mean absolute and relative testes weights were significantly reduced at 300 mg/kg bw/d and these testicular findings were associated histopathologically with interstitial cell atrophy and the depletion of mature germ cells; interstitial cell atrophy was also noted in all males dosed at 180 mg/kg bw/d. There were marked reductions in mean absolute epididymal weights in males dosed at 180 and 300 mg/kg bw/d with relative epididymal weights also significantly lower. Histopathology revealed epididymal hypospermia and the presence of luminal debris in the majority of 300 mg/kg bw/d males. Treatment-related effects on the female reproductive tract were also apparent at 180 and 300 mg/kg bw/d at Week 4 with reduced ovary weights accompanied by decreased numbers of corpora lutea. Effects on the male reproductive tract were considered to be a direct consequence of the systemic toxicity observed at these dose levels. Specifically, the effects on the male reproductive tract at 180 and 300 mg/kg bw/d observed in this study can be explained by the markedly reduced weight gain (55-58% of controls) of males at 180 and 300 mg/kg bw/d during the dosing period with mean terminal bodyweights comparable to those of controls at Week 2. Since the rats were eight weeks old at the start of the study, the terminal bodyweights of males at 180 and 300 mg/kg bw/d were therefore equivalent to those of 10-week old control rats. It is known that male rats attain sexual maturation at around 8-10 weeks of age but that maturation is also dependent on bodyweight, therefore the severe bodyweight effects resulting from administering TPP at 180 and 300 mg/kg bw/d are considered sufficient to have resulted in delaying sexual maturation among these animals.

A 90-day dietary toxicity study in the rat (Haas, 2007), identified a NOAEL of <50 mg/kg bw/d, based on bodyweight effects. No deaths occurred and no clinical signs were observed during the study period although systemic toxicity as evidenced by reductions in absolute body weight and body weight gain, were observed at all treatment levels. Animals dosed at ≥100 mg/kg bw/day also showed reduced food consumption, an effect attributable to toxicity rather than palatability of the diet as rats are known to readily adapt to unpalatable diets and similar reductions in food consumption were observed in other studies where animals dosed by gavage. Other indicators of systemic toxicity included, reductions in red blood cell counts and haemoglobin in males at 200 mg/kg bw/d, and lower white blood cell counts and lymphocyte counts in either sex at 200 mg/kg bw/d, increased mean absolute adrenal weights in males dosed at ≥150 mg/kg bw/d, and increased relative adrenal weights in either sex at this dose level or higher, and among males dosed

at ≥100 mg/kg bw/d (suggestive of a stress response). Animals dosed at ≥ 150 mg/kg bw/d also showed periportal hepatocellular vacuolization. Gross necropsy revealed small testes, epididymides, coagulating gland, prostate and seminal vesicles at 200 mg/kg bw/d; small coagulating gland, prostate and seminal vesicles were also observed at 150 mg/kg bw/d. Mean lower absolute and relative (to bodyweight and brain weight) weights of the epididymides, prostate and seminal vesicles were observed in males at ≥100 mg/kg bw/d. Mean lower absolute and higher relative testes weights were observed at 200 mg/kg bw/d; mean relative (to brain weight) testes weights were significantly reduced in males at 200 mg/kg bw/d only. Additionally, mean lower absolute and relative (to bodyweight and brain weight) ovary weights were observed in females at these dose levels. Mean lower testes weight (relative to brain weight) was observed in males at 200 mg/kg bw/d only. Organ weight effects were accompanied by histopathology findings of coagulating gland atrophy and prostate atrophy in males at 200 mg/kg bw/d, decreased secretion in the seminal vesicles in males at ≥150 mg/kg bw/d and decreased corpora lutea in the ovaries in females at ≥150 mg/kg bw/d. There was, therefore, no evidence of an adverse effect of TPP on the reproductive tract that was not accompanied by significant general systemic toxicity, especially reduced bodyweight gain.

An older 90-day rat dietary toxicity study (Vogin, 1970) identified a NOAEL of 100 mg/kg bw/d. 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/d in males and females. Mean absolute and relative testes weights were reduced in males dosed at 200 mg/kg bw/d with testicular hypospermia observed in six out of 20 animals. Additionally, liver weights were increased among either sex dosed at 200 mg/kg bw/d. No additional histopathological effects were noted in this study.

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. All studies were conducted using oral administration, generally via dietary inclusion.

4.7.1.5 Human information

No data are available.

4.7.1.6 Other relevant information

No data are available.

4.7.1.7 Summary and discussion of repeated dose toxicity

The results of repeated dose toxicity studies performed in the rat using tetrapropenylphenol are consistent in identifying effects on the male reproductive tract. Findings are characterised by

reduced weights of the prostate, seminal vesicles, epididymides and seminal vesicles and (at high dose levels) the testes. Effects on organ weights are associated microscopically with prostatic hypoplasia and reduced secretion, epididymal hypoplasia and hypospermia, seminal vesicle hypoplasia and hypospermia and coagulating gland atrophy. Effects on the testes at high dose levels were associated with interstitial cell atrophy, hypospermia and maturation depletion. Effects on the male reproductive tract in all studies are accompanied by general toxicity (clinical signs, bodyweight effects); effects at high dose levels in some studies are associated with relatively marked general toxicity and may be secondary to general toxicity, representing a developmental delay. No effects on the male reproductive tract were observed in a 90-day dog study.

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

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

STOT-RE is assigned on the basis of findings of 'significant' or 'severe' toxicity. 'Significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. 'Severe' effects are generally more profound or serious than 'significant' effects and are of a considerably adverse nature which significantly impact on health. The effects observed in the battery of repeated administration tests completed for phenol, dodecyl-, branched were limited to microscopic changes in reproductive organs and associated organ weight changes.

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

Substances are classified as specific target organ toxicants following repeated exposure by the use of expert judgement, on the basis of the weight of all evidence available, including the use of recommended guidance values which take into account the duration of exposure and the dose/concentration which produced the effect(s), and are placed in one of two categories, depending upon the nature and severity of the effect(s) observed.

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

There were no changes observed in any of the test species that indicated effects considered to constitute clear functional disturbance, serious or significant toxic changes to specific organs at dose levels below the relevant cut-off points. The changes observed in sub-acute or sub-chronic exposure studies were addressed according to criteria for hazardous properties and the appropriate NOAEL identified. None of the target organs were affected at sub-toxic doses and none of the effects warrants classification as STOT-RE.

None of the observed changes are considered to be significantly or severely adverse and therefore do not trigger the classification of phenol, dodecyl-, branched for STOT-RE according to Regulation (EC) No. 1272/2008. The available data do not indicate classification of the substance for repeated dose toxicity according to CLP criteria. However the findings in rat studies of effects on the male reproductive tract warrant further consideration against the criteria for reproductive toxicity classification. The results of the repeated dose toxicity studies are therefore considered in detail in the reproductive toxicity section.

Justification for classification or non classification

The key parameters chosen for repeated dose toxicity for the oral route were considered to be greater than the criteria set out in Regulation (EC) No. 1272/2008, therefore classification for repeated dose toxicity (STOT-RE) is not considered to be necessary.

The key parameter chosen for the 28 day repeated dose toxicity: oral study, gave a NOAEL of 60 mg/kg bw/d, effects were seen at 180 and 300 mg/kg bw/d, however these are above the cut off values for effects seen.

The key parameter chosen for the 90 day repeated dose toxicity: oral study, gave a NOAEL of 25 mg/kg bw/d, effects were seen at 100 and 200 mg/kg bw/d, however these are above the cut off values for effects seen.

Classification for repeated dose toxicity is not required according to DSD criteria.

4.9 Germ cell mutagenicity (Mutagenicity)

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

Method	Results	Remarks	Reference
***In vitro* tests**			
Bacterial reverse mutation assay (Ames test-gene mutation) Doses: 0.1, 0.33, 1.0, 3.33 and 10.0 mg/plate with and without activation Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Negative Negative for *S. typhimurium* TA1535, TA1537, TA98 and TA100 (all strains/cell types tested with or without metabolic activation; cytotoxicity-yes	Experimental result Test material: Phenol, (tetrapropenyl) derivatives (CAS No. 74499-35-7) Test material purity not provided.	Machado *et al* (1989)
Bacterial reverse mutation assay (Ames test-gene mutation) Doses: 8, 40, 200, 1000 and 5000 µg/plate with and without metabolic activation, with and without pre-incubation EU Method B.13/14 (Mutagenicity-Reverse Mutation Test Using Bacteria)	Negative Negative for *S. typhimurium* TA1535, TA1537, TA98 and TA100 (all strains/cell types tested); with and without metabolic activation Negative for *S. typhimurium* TA1538; with and without metabolic activation (pre-incubation test)	Experimental result Test material: Phenol, dodecyl -, branched (CAS No. 121158-58-5) 100%.	Schörberl P (1992b) OECD SIDS(2006)
Bacterial reverse mutation assay (Ames test-gene mutation) Doses: 1.0 to 1000 µg/plate Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Negative Negative for *S. typhimurium* TA1535, TA1537, TA98 and TA 100 (all strains/cell types tested); with and without metabolic activation	Experimental result Test material: Phenol, dodecyl-, branched: further details not reported	Anon (2006) Condray (1987)
Mammalian cell gene mutation assay -Chinese hamster ovary (CHO) Doses: up to 10 µg/mL in the presence of metabolic activation system and up to 0.1 µg/mL in the absence of metabolic activation system. Equivalent or similar to OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)	Negative Negative for Chinese hamster ovary (CHO) (all strains/cell types tested with or without metabolic activation)	Experimental result Test material: Phenol, dodecyl-, branched further details not reported	Anon (2006)
***In vivo* tests**			
Micronucleus assay (chromosome aberration in rats) at dose levels of 0, 500, 1500 & 5000 mg/kg bw equivalent or similar to OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	Negative Genotoxicity: negative	Experimental result Test material: Phenol, dodecyl-, branched further details not reported	Condray (1987) OECD SIDS(2006)

4.9.1 Non-human information

4.9.1.1 *In vitro* data

Phenol, dodecyl-, branched was tested in an appropriate battery of *in vitro* studies including several bacterial reverse mutation assays and a mammalian cell gene mutation assay. The results were all negative for mutagenic potential, with or without metabolic activation. The overall assessment is that phenol, dodecyl-, branched is not considered to be genotoxic; this was confirmed by the negative results of an *in vivo* micronucleus test.

4.9.1.2 *In vivo* data

The *in vivo* assessment of micronucleus induction in rat erythrocytes gave a negative response for mutagenic potential.

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

In vitro studies

Ames test: in the key study for *in vitro* genetic toxicity (Machado *et al*, 1989) there was no guideline specified, however it was considered to be comparable to OECD Guideline 471 (Bacterial Reverse Mutation Assay). The study was conducted in line with GLP. The test material was diluted with 25% Pluronic F127 (w/w in ethanol) and tested in the histidine-deficient strains of *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 at concentrations of 0.1-10 mg/plate with and without S-9 metabolic activation. The test material appeared to form a stable emulsion with Pluronic F127 and the dilutions were well dispersed in the top agar; however, after incubation, test material was observed on the surface of the top agar at 10 mg/plate. The test material was cytotoxic at 10 mg/plate to strain TA100 with and without S-9 and at >3.3 mg/plate to TA1535 with S-9. No statistically significant increases in mutant frequency were observed in any strain. Under the conditions tested, the test material was not mutagenic to strain TA98, TA100, TA1535, or TA1537 with or without metabolic activation. A supporting study (Schörberl, 1992; Ames test), is also available for this endpoint. Under the conditions of this study, the substance was not mutagenic. A further supporting study (Condray, 1987, Ames test) is also available for this endpoint. No statistically significant increases in mutation frequency were observed at dose levels of 1.0-1000 μg/plate in all bacterial strains tested with and without an S-9 metabolic activation system.

Mammalian Cell Gene Mutation Test: in the key study for *in vitro* genetic toxicity (Condray, 1987) there was no guideline specified, however it was considered to be comparable to OECD Guideline 476 (*In vitro* Mammalian Cell Gene Mutation Test). The substance was not mutagenic when tested in the CHO/HGPRT forward mutation assay at concentrations up to 10 μg/mL in the presence of S-9 metabolic activation and up to 0.1 μg/mL in the absence of S-9.

In vivo study

The key study for *in vivo* genetic toxicity (Condray, 1987) was considered to be comparable to OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test). Mortality was observed at the high dose level, and reduced body weight gain was observed in the mid- and high-dose groups. There was no evidence of chromosome damage as measured by increases in chromosome aberrations, altered mitotic index, or chromosome number when compared to the concurrent control group. In the cyclophosphamide-treated positive control group, a significant increase in the average number of aberrations, percent of cells with aberrations, and decreased mitotic index was observed confirming the sensitivity of the assay. Under the conditions of this study the test material is not clastogenic.

Justification for classification or non classification

The results for the key parameters chosen for genetic toxicity were negative and so the criteria set out in Regulation (EC) no 1272/2008 do not apply, therefore classification for genetic toxicity is not considered to be necessary.

4.9.5 Comparison with criteria

Phenol, dodecyl-, branched was tested in a battery of *in vitro* and *in vivo* assays without displaying any signs of mutagenic activity. Based on the results, no classification for mutagenicity is required according to CLP or DSD criteria.

4.9.6 Conclusions on classification and labelling

Phenol, dodecyl-, branched was concluded to be non-genotoxic based on the results of studies *in vitro* and *in vivo*, and consequently no classification for potential mutagenic risk is required according to CLP or DSD criteria.

4.10 Carcinogenicity

Table 23: 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 studies are available.

4.10.1.1 Carcinogenicity: oral

No studies are available.

4.10.1.2 Carcinogenicity: inhalation

No study data are available for exposure via the inhalation route.

4.10.1.3 Carcinogenicity: dermal

No studies are available for exposure via the dermal route.

4.10.2 Human information

No information are available

4.10.3 Other relevant information

No other relevant information is available.

4.10.4 Summary and discussion of carcinogenicity

No studies are available determination of carcinogenic potential. There are no indications of genotoxicity and genotoxic carcinogenicity is considered unlikely for the substance.

4.10.5 Comparison with criteria

In the absence of experimental data on carcinogenicity and no indication of mutagenicity, classification for carcinogenicity is not required according to DSD criteria.

4.10.6 Conclusions on classification and labelling

No classification for potential carcinogenicity is indicated for phenol, dodecyl-, branched.

In the absence of any evidence of carcinogenicity in animal studies, phenol, dodecyl-, branched does not fulfil the criteria for classification as a Category 1 or 2 carcinogen under the CLP Regulation (EC 1272/2008). Classification for carcinogenicity is not required according to DSD criteria.

4.11 Toxicity for reproduction

Table 25: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Two-generation reproductive toxicity study in rats (Sprague-Dawley) male/female by oral dietary exposure at dose levels of 0, 1.5, 15 & 75 mg/kg bw/d (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 to the day of euthanasia. The F0 and F1 females continued to receive the control and test diets throughout mating, gestation, and lactation through to 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 diet (male/female) based on: test material-overall effects, body weight; food consumption, organ weights; and histopathology. **NOAEL (Parental toxicity) (F1):** 1.5 mg/kg diet (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/d (nominal) (male/female) based on: test material. Based on decreased implantation sites, increased oestrous cycle lengths and a reduction in mean epididymal sperm concentration. **NOAEL (Neonatal toxicity) (F1 and F2):** 15 mg/kg bw/d (nominal) (male/female) based on: test material. 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.)	Key study experimental result Test material: tetrapropenyl phenol (CAS 74499-35-7); 100% purity	Edwards TL (2010)
One-generation reproductive toxicity study in rats (Sprague-Dawley) male/female by oral gavage administration at doses of 0, 5, 25 & 125 mg/kg bw/d (actual 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	**NOAEL (P):** <5 mg/kg bw/d (male/female) based on: test mat. (Reproductive toxicity) **NOAEL (P):** 5 mg/kg bw/d (male/female) based on: test material. (Systemic toxicity) **NOAEL (F1):** 5 mg/kg bw/d (male/female) based on: test material. (Neonatal toxicity)	Supporting study experimental result. Test material: tetrapropenyl phenol (CAS 74499-35-7); 100% purity	Knapp JF (2006)

Method	Results	Remarks	Reference
the day prior to euthanasia, for a total of 115 to 128 doses. (Once daily) OECD Guideline 415 (One-Generation Reproduction Toxicity Study)			
Developmental toxicity			
Pre-natal developmental study by oral gavage administration to rats (Sprague-Dawley) at dose levels of 0, 20, 100 and 300 mg/kg bw/d (actual ingested) 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/d, actual dose received based on test material-Phenol, dodecyl-, branched. **NOAEL (embryotoxicity):** 100 mg/kg bw/d actual dose received based on test material-Phenol, dodecyl-, branched. **NOAEL (foetotoxicity):** 100 mg/kg bw/d actual dose received based on test material-Phenol, dodecyl-, branched. **NOAEL (teratogenicity):** 100 mg/kg bw/d actual dose received based on test material-Phenol, dodecyl-, branched.	Experimental result Test material: phenol (tetrapropenyl derivatives) CAS 27193-86-8: 100% purity	Schroeder, R (1987) Schroeder, R (1985)

4.11.1 Effects on fertility

Relevant data are extracted from the repeated dose systemic and reproductive toxicity studies performed with TPP. Data are summarised and compared against the criteria for classification for reproductive toxicity under the CLP Regulation, and published opinions of the Committee for Risk Assessment (RAC) concerning substances classified for reproductive effects. Based on a consideration of all of these factors it is concluded that TPP is most appropriately classified under CLP Regulation as:

Reproductive toxicity (adverse effects on sexual function and fertility): Category 2

Reproductive toxicity (adverse effects on development of the offspring): Not classified

TPP only affected sexual function and fertility at doses that resulted in general systemic toxicity. At doses below the threshold of systemic toxicity, effects on sexual function and fertility were not observed. Adverse effects of TPP on reproduction function were not seen in a definitive 2-generation dietary study. Deficiencies in the quality of the findings in rats due to the likelihood of surpassing hepatic metabolic capacity as a result of oral bolus gavage dosing, and the lack of reproducible findings in alternative species (i.e., dog) calls into question relevance of the rat findings to humans. In this case a Category 2 classification is more appropriate for TPP, which would be consistent with previous RAC decisions.

4.11.1.1 Non-human information

a) Repeated dose toxicity studies

i. 14-day rat gavage toxicity study [Harriman, 2003; WIL Research Laboratories, WIL-186031]

In this range-finding study, groups of CD rats (3/sex) were treated with TPP (in corn oil) by oral bolus gavage at dose levels of 0 (vehicle controls), 30, 75, 150, 300 and 500 mg/kg bw/d, daily for 14 days. A summary of relevant findings is shown in Table 26.

Treated animals showed a spectrum of systemic toxicity including a) mortality at 500 mg/kg bw/d, b) clinical observations of perianal staining, hair loss, soft faeces, clear yellow/brown material on body surfaces and unkempt appearance at doses ≥150 mg/kg bw/d, c) body weight (absolute and gain) reductions among males at doses ≥ 300 mg/kg bw/d and d) an increase in adrenal weights (suggestive of a stress response) at doses ≥ 300 mg/kg bw/d.

Findings of relevance to reproductive toxicity

Gross necropsy revealed small prostate and seminal vesicles in the two surviving 500 mg/kg bw/d males; small seminal vesicles were also observed in one male at 300 mg/kg bw/d. Reduced absolute and relative weights of the prostate, seminal vesicles and epididymides were observed in males at ≥150 mg/kg bw/d. Absolute testes weights were lower in these dose groups however relative weights were comparable to controls, indicating that these effects were secondary to reduced bodyweight. No treatment-related findings were reported for the female reproductive tract in this study.

Summary: This oral bolus gavage study resulted in clear general systemic toxicity to the experimental animals at doses that also resulted in effects on reproductive organ weights. The relevance of bolus dosing studies such as this is questionable since it has been shown that alkyl phenols saturate hepatic detoxification mechanisms in the rat at doses approaching 100 mg/kg (Certa, et al., 1996). Thus the toxicity profile of the test substance at doses approaching this level and above is artificially exaggerated by the method of administration, and in the case of TPP this toxicity results in secondary effects on reproductive function.

A NOAEL of 75 mg/kg bw/d was assigned both for general systemic toxicity and for effects on the male reproductive tract.

Table 26: 14-day rat study: summary of relevant findings

Shading illustrates the doses of TPP that resulted in evidence of both systemic toxicity and effects on reproductive organ weights.

Observation	Sex	Dose level (mg/kg bw/d)					
		0	30	75	150	300	500
Mortality (#)	**M**	-	-	-	-	-	1
	F	-	-	-	-	-	-
Adverse clinical signs	**M**	-	-	-	✔	✔	✔
	F	-	-	-	✔	✔	✔
Terminal bodyweight (g)	**M**	299	307	313	281	276	261
	F	210	207	202	202	218	230
Weight gain (g): **Week 0-1**	**M**	31	35	40	27	15	-14**
	F	12	10	7	11	14	16
Weight gain (g)**: Week 0-2**	**M**	67	72 [107%]	81 [121%]	49 [73%]	42 [63%]	30 [45%]
	F	29	28	20	23	40	50*
Prostate small (#)	**M**	-	-	-	-	-	2
Seminal vesicles small (#)	**M**	-	-	-	-	1	2
Prostate weight (g)	**M**	0.72	0.55 [76%]	0.73 [101%]	0.37 [51%]	0.34* [47%]	0.32 [44%]
Prostate weight (%)		0.239	0.179 [75%]	0.238 [100%]	0.132 [55%]	0.122 [51%]	0.121 [51%]
Seminal vesicles weight (g)	**M**	1.09	1.23 [113%]	0.94 [86%]	0.58** [53%]	0.53** [49%]	0.27 [25%]
Seminal vesicles weight (%)		0.364	0.402 [110%]	0.303 [83%]	0.205* [56%]	0.193** [53%]	0.104 [29%]
Epididymides weight (g)	**M**	0.79	0.77 [97%]	0.76 [96%]	0.69* [87%]	0.62** [78%]	0.50 [63%]
Epididymides weight (%)		0.263	0.252 [96%]	0.243 [92%]	0.246 [94%]	0.226 [86%]	0.190 [72%]
Testes weight (g)	**M**	3.04	2.84 [93%]	2.93 [96%]	2.83 [93%]	2.75 [90%]	2.55 [84%]
Testes weight (%)		1.007	0.926 [92%]	0.938 [93%]	1.006 [100%]	1.001 [99%]	0.978 [97%]

**significantly different to controls ($p<0.05$); **$p<0.01$. # = number of animals.*

ii. 28-day dietary rat toxicity study (Reyna & Thake, 1988; Monsanto ML-87-041)

Sprague-Dawley rats were administered TPP at dietary concentrations of 0 (controls), 500, 2500 and 5000 ppm, equivalent to intakes of approximately 0, 40, 180 and 300 mg/kg bw/d for 28 consecutive days. A summary of relevant findings is shown in Tables 27 and 28.

No deaths occurred during the study period, however evidence for systemic toxicity included a) reduced food consumption of 27-49%, weight loss or reduced weight gain at the highest dose level in either sex in this study, with effects on both food consumption and weight gain also apparent at 180 mg/kg bw/d, b) red-coloured urine was observed in either sex at 300 mg/kg bw/d and in

females at 180 mg/kg d and c) increased adrenal weights in males (suggestive of a stress response) at 300 mg/kg bw/d. Liver weights were also increased in females in this group.

Findings of relevance to reproductive toxicity

Gross necropsy revealed treatment-related changes in the reproductive tract (small/atrophic prostate and seminal vesicles, small and/or soft testes) in eight males at 300 mg/kg bw/d. Findings were confirmed histopathologically in seven of these rats and consisted of epididymal hypoplasia and hypospermia, reduced prostatic secretion and prostatic hypoplasia, reduced seminal vesicle secretion and seminal vesicle hypoplasia. Effects of treatment were therefore apparent on the male reproductive tract in this study at 300 mg/kg bw/d. No effects on the male reproductive tract were reported at 180 mg/kg bw/d, a dose level at which less marked systemic toxicity was apparent. No treatment-related findings were reported for the female reproductive tract in this study.

Summary: In this repeated dose dietary study, TPP produced general systemic toxicity in the rat at doses significantly higher that observed in bolus dosing studies, supporting the notion that oral bolus gavage is an inappropriate route for assessing the reproduction toxicity of TPP due to the potential for exceeding the capacity of hepatic first-pass detoxification mechanisms. Further, this study once again shows the direct correlation between systemic toxicity and effects on reproduction parameters (in this case testes weight) and the absence of reproduction effects at doses that result in less systemic toxicity.

A NOAEL at 180 mg/kg bw/d (2500 ppm) was assigned for general systemic toxicity and at 300 mg/kg bw/d (5000 ppm) for effects on the male reproductive tract.

Table 27: 28-day rat gavage study: summary of relevant findings

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

Observation	Sex	Dose level (mg/kg bw/d)			
		0 (0 ppm)	40 (500 ppm)	180 (2500 ppm)	300 (5000 ppm)
Body Weight Gain (g)	M	115.2	124.2	40.9	-15.5
	F	39.1	30.5	18.5	11.1
Terminal Body Weight (g)	M	419.4	425.5	345.4**	283.7**
	F	199.2	194.0	176.1**	180.1**
Adrenal Gland Weight (g)	M	0.077	0.082	0.089	0.092*
	F	0.086	0.090	0.082	0.074*
Mean Kidney Weight (g)	M	3.57	3.98	3.16	2.62 **
	F	1.09	1.74	1.65 *	1.61 **
Liver Weight (g)	M	14.2	15.2	12.5	11.4 **
	F	6.4	6.5	6.5	7.3 **
Testes Weight (g)	M	4.77	5.66	4.51	3.27 **

**significantly different to controls ($p<0.05$); **$p<0.01$.*

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

Shading illustrates the doses of TPP that resulted in evidence of both systemic toxicity and effects on vital and reproductive organs.

Observation	Sex	Dose level (mg/kg bw/d)			
		0	40 (500 ppm)	180 (2500 ppm)	300 (5000 ppm)
Liver – periportal vacuolization	**M**	-	NE	NE	3 (1-2)
	F	-	NE	NE	1 (1)
Kidney – mineralization	**M**	1 (1)	NE	NE	2 (1)
	F	2 (1)	NE	NE	1 (1)
Bone Marrow – hypoplasia	**M**	-	-	-	6 (1)
	F	-	-	-	3 (1)
Spleen – congestion	**M**	-	-	1 (1)	4 (1)
	F	-	-	-	5 (1)
Testes – tubular hypoplasia	**M**	-	-	-	4 (3-5)
Epidydimides – decreased or absent sperm	**M**	1 (5)	-	-	4 (4-5)
Epidydimides – hypoplasia	**M**	-	-	-	1 (4)
Seminal Vesicles – absence of secretions	**M**	-	-	-	5 (5)
Prostate – abnormal or absent secretions	**M**	-	-	-	7 (2-5)
Prostate – hypoplasia	**M**	1 (3)	-	-	4 (3-5)

 # = number of animals.

iii. 28-day gavage rat toxicity study [Harriman, 2004; WIL Research Laboratories, WIL-186032]

Groups of CD rats (5/sex) were treated with TPP (in corn oil) by oral bolus gavage at dose levels of 0 (vehicle controls), 5, 20, 60, 180 and 300 mg/kg bw/d for 28 days. Additional groups of rats were dosed at 0 and 300 mg/kg bw/d for 28 days followed by a 14-day recovery period. A summary of relevant findings is shown in Tables 29 and 30.

No deaths occurred during the course of the study. However, general systemic toxicity was evident through a) clinical signs (excessive salivation and perianal staining) at dose levels of ≥60 mg/kg bw/d, b) markedly reduced bodyweights, weight gains and food consumption were seen in males at ≥180 mg/kg bw/d, c) increased adrenal weights (suggestive of a stress response) at ≥180 mg/kg bw/d, d) haematology effects of reduced haemoglobin and haematocrit, reduced lymphocyte counts, and increased reticulocyte counts in females at ≥180 mg/kg bw/d and e) centrilobular hepatocyte hypertrophy and hepatocyte vacuolization in the liver, and hypertrophy of follicular cells of the thyroid in males.

<u>Findings of relevance to reproductive toxicity</u>

At Week 4, gross necropsy revealed small testes, prostate, seminal vesicles, epididymides and/or coagulating gland in males at ≥180 mg/kg bw/d. Mean absolute and relative weights of the seminal vesicles were markedly reduced at 180 and 300 mg/kg bw/d and reduced seminal vesicle secretion was observed in all males at both dose levels. Mean absolute and relative prostate weights were also markedly reduced in these animals together with reduced prostatic secretion. Mean absolute and relative testes weights were significantly reduced at 300 mg/kg bw/d and these testicular findings were associated histopathologically with interstitial cell atrophy and the depletion of

mature germ cells; interstitial cell atrophy was also noted in all males dosed at 180 mg/kg bw/d. There were marked reductions in mean absolute epididymal weights in males dosed at 180 and 300 mg/kg bw/d with relative epididymal weights also significantly lower. Histopathology revealed epididymal hypospermia and the presence of luminal debris in the majority of 300 mg/kg bw/d males. Treatment-related effects on the female reproductive tract were also apparent at 180 and 300 mg/kg bw/d at Week 4 with reduced ovary weights accompanied by decreased numbers of corpora lutea.

Effects on the male reproductive tract were considered to be a direct consequence of the systemic toxicity observed at these dose levels. Specifically, the effects on the male reproductive tract at 180 and 300 mg/kg bw/d observed in this study can be explained by the markedly reduced weight gain (55-58% of controls) of males at 180 and 300 mg/kg bw/d during the dosing period with mean terminal bodyweights comparable to those of controls at Week 2. Since the rats were eight weeks old at the start of the study, the terminal bodyweights of males at 180 and 300 mg/kg bw/d were therefore equivalent to those of 10-week old control rats. It is known that male rats attain sexual maturation at around 8-10 weeks of age but that maturation is also dependent on bodyweight, therefore the severe bodyweight effects resulting from administering TPP at 180 and 300 mg/kg bw/d are considered sufficient to have resulted in delaying sexual maturation among these animals.

Recovery period

After the 14 day off-treatment period, reduction in mean body weight gain had partially recovered and was no longer statistically significant; adrenal weights were similar to controls; effects of reduced haemoglobin and haematocrit and reduced lymphocyte counts were no longer apparent; and the liver and thyroid histopathology findings were similar to control. Effects of treatment on the male reproductive tract were observed at dose levels of 180 and 300 mg/kg bw/d. were shown to be largely reversible within 14 days of the withdrawal of treatment indicating that the reproductive effects are secondary to treatment-related systemic toxicity. Effects on accessory glands at 300 mg/kg bw/d had completely resolved following the recovery period. Seminal vesicle weights at 300 mg/kg bw/d remained lower after the recovery period, but after 14 days reached recovery to approximately 80% of control values. However, the reduced seminal vesicle secretion observed at Week 4 was not observed in most of the males following the recovery period. Effects on the prostate resolved following the recovery period and the reduced prostatic secretion observed at Week 4 was not apparent at the end of the recovery period. The reduced mean absolute and relative testes weights evident at Week 4 were also absent following recovery and there were no significant effects on epididymal weights following the recovery period. Following the recovery period treatment-related effects on the female reproductive tract were mostly resolved and were observed in a single 300 mg/kg bw/d female.

Summary: The results of this repeated dose oral gavage study in rats once again highlights the greater intensity of systemic toxicity resulting from the bolus method of test substance administration compared to results found with dietary studies. Secondary to these general systemic toxicity effects, one finds a gradual increase number of significant effects on reproductive organ parameters as a function of dose and the corresponding increase in the degree of systemic toxicity. The findings at the end of the recovery period clearly show that when the systemic toxicity induced by treatment with TPP dissipates following cessation of treatment, the effects on reproduction parameters rapidly resolve as well. This phenomenon adds further support to the argument that the effects of TPP on reproduction organs occur secondary to general systemic toxicity.

A NOAEL of 60 mg/kg bw/d was assigned for general systemic toxicity and for effects on the male reproductive tract.

Table 29: 28-day rat gavage study: summary of relevant findings

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

Observation	Sex	Dose level (mg/kg bw/d)					
		0	**5**	**20**	**60**	**180**	**300**
Adverse clinical signs	**M**	-	-	-	-	✔	✔
	F	-	-	-	✔	✔	✔
Terminal bodyweight (g)	**M**	365	374	362	363	319*	327*
	F	237	224	236	230	223	232
Weight gain (g)	**M**	100	104	103	95	55**	58**
	F	42	33	40	35	33	36
Liver (g)	**M**	8.90	9.85	9.76	10.50	10.66	12.25**
	F	6.48	6.19	6.75	6.66	7.81	8.90**
Adrenals (g)	**M**	0.0533	0.0605	0.0665	0.0629	0.0900**	0.1017**
	F	0.0746	0.0775	0.0850	0.0689	0.0926	0.0908
Adrenals (%)	**M**	0.016	0.017	0.020	0.019	0.031**	0.034**
	F	0.034	0.037	0.038	0.032	0.040	0043*
Adrenals (g/100 g brain)	**M**	2.72	3.02	3.44	3.18	4.63**	5.32**
	F	4.06	4.26	4.24	3.88	4.46	4.92
Seminal vesicles (g)	**M**	1.49	1.47	1.74	1.36	0.49**	0.32**
Seminal vesicles (%)		0.446	0.420	0.513	0.404	0.170**	0.109**
Seminal vesicles (g/100 g brain)		76.1	73.5	90.4	68.8	25.7**	16.6**
Prostate (g)		0.79	0.83	0.72	0.788	0.35**	0.17**
Prostate (%)		0.236	0.237	0.214	0.230	0.118**	0.057**
Prostate (g/100 g brain)		40.4	41.5	36.9	39.5	17.9**	8.57**
Testes (g)		3.35	3.13	3.27	3.28	2.85	1.93**
Testes (%)		1.001	0.897	0.965	0.979	0.978	0.647**
Testes (g/100 g brain)		171.6	155.9	169.1	166.0	148.1	100.1**
Epididymides (g)		1.16	1.01	10.9	1.18	0.93**	0.49**
Epididymides (%)		0.347	0.288	0.323	0.351	0.286	0.165**
Epididymides (g/100 g brain)		59.2	50.2	56.4	59.9	43.3*	25.5**
Ovary (g)	**F**	0.1379	0.1271	0.1336	0.1219	0.1047*	0.0992*
Ovary (%)		0.062	0.061	0.060	0.057	0.051	0.047*
Ovary (g/100 g brain)		7.53	7.00	6.72	6.87	5.63	5.36

**significantly different to controls ($p<0.05$); **$p<0.01$.*

Table 30: 28-day rat gavage study: summary of pathology

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

Observation	Sex	Dose level (mg/kg bw/d)					
		0	5	20	60	180	300
Liver – centrilobular hepatocellular hypertrophy	M	0/5	-	0/5	2/5 (1)	2/5 (1)	5/5 (2)
	F	0/5	-	-	0/5	4/5 (1)	5/5 (1-2)
Liver – periportal hepatocellular vacuolization	M	0/5	-	0/5	0/5	0/5	3/5 (1-3)
	F	0/5	-	NE	0/5	0/5	1/5 (1)
Prostate: small (#)	M	-	-	-	-	1	3 (0)
Seminal vesicles: small(#)		-	-	-	-	4	4 (0)
Testes: small(#)		-	1	-	-	-	2 (0)
Coagulating gland: small(#)		-	-	-	-	2	4 (0)
Epididymides small(#)		-	1	-	-	-	2 (0)
Epididymal hypospermia		-	-	-	-	-	3 (1)
Epididymal debris		-	-	-	-	-	4 (1)
Prostate: decreased secretion		-	-	-	-	5	5 (0)
Seminal vesicles: decreased secretion		-	-	-	-	5	5 (1)
Testes: maturation depletion		-	-	-	-	1	4 (1)
Testes: interstitial cell atrophy		-	-	-	-	5	4 (0)
Coagulating gland: decreased secretion		-			-	5	5 (0)
Decreased corpora lutea	F	-	-	-	-	2	3 (1)

 # = number of animals. Values in brackets () at 300 mg/kg bw/day are values after the 14 day recovery period.

iv. 90-day rat dietary toxicity study (Haas, 2007); WIL Research Laboratories, WIL-186054

Groups of CD rats (10/sex) were administered TPP in the diet at dose levels equivalent to 0 (controls), 50, 100, 150 and 200 mg/kg bw/d. Dietary concentrations were adjusted weekly, based on predicted bodyweight and food consumption. A summary of relevant findings is shown in Table 31. No deaths occurred and no clinical signs were observed during the study period although systemic toxicity as evidenced by reductions in absolute body weight and body weight gain, were observed at all treatment levels. Animals dosed at ≥100 mg/kg bw/day also showed reduced food consumption, an effect attributable to toxicity rather than palatability of the diet as rats are known to readily adapt to unpalatable diets and similar reductions in food consumption were observed in other studies where animals dosed by gavage. Other indicators of systemic toxicity included, reductions in red blood cell counts and haemoglobin in males at 200 mg/kg bw/d, and lower white blood cell counts and lymphocyte counts in either sex at 200 mg/kg bw/d, increased mean absolute adrenal weights in males dosed at ≥150 mg/kg bw/d, and increased relative adrenal weights in either sex at this dose level or higher, and among males dosed at ≥100 mg/kg bw/d (suggestive of a stress response). Animals dosed at ≥150 mg/kg bw/d also showed periportal hepatocellular vacuolization.

Findings of relevance to reproductive toxicity

Gross necropsy revealed small testes, epididymides, coagulating gland, prostate and seminal vesicles at 200 mg/kg bw/d; small coagulating gland, prostate and seminal vesicles were also observed at 150 mg/kg bw/d. Mean lower absolute and relative (to bodyweight and brain weight) weights of the epididymides, prostate and seminal vesicles were observed in males at ≥100 mg/kg bw/d. Mean lower absolute and higher relative testes weights were observed at 200 mg/kg bw/d; mean relative (to brain weight) testes weights were significantly reduced in males at 200 mg/kg bw/d only. Additionally, mean lower absolute and relative (to bodyweight and brain weight) ovary weights were observed in females at these dose levels. Mean lower testes weight (relative to brain weight) was observed in males at 200 mg/kg bw/d only. Organ weight effects were accompanied by histopathology findings of coagulating gland atrophy and prostate atrophy in males at 200 mg/kg bw/d, decreased secretion in the seminal vesicles in males at ≥150 mg/kg bw/d and decreased corpora lutea in the ovaries in females at ≥150 mg/kg bw/d. There was, therefore, no evidence of an adverse effect of TPP on the reproductive tract that was not accompanied by significant general systemic toxicity, especially reduced bodyweight gain.

Summary: The results of this repeated dose study clearly demonstrate the direct correlation between general systemic toxicity and effects on reproductive organs parameters. Cumulative general systemic toxicity resulting from the extended treatment period (90 days) becomes evidence at 50 mg/kg bw/d before evidence of reproductive effects manifest. The secondary effect on reproduction becomes clear as the intensity of systemic toxicity increases at higher doses, and only then do the effects on reproduction parameters become evident.

A NOAEL for general toxicity cannot be determined for this study however due to bodyweight effects observed at all dose levels. A NOAEL of 50 mg/kg bw/d was assigned for effects on the reproductive tract of either sex.

Table 31: 90-day rat study: summary of findings

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

Observation	Sex	Dose level (mg/kg bw/d)				
		0	**50**	**100**	**150**	**200**
Bodyweight (g)	**M**	524	465**	433**	372**	342**
	F	299	272*	265**	261**	246**
Weight gain (g)	**M**	311	252**	220**	159**	129**
	F	129	103**	96**	91**	76**
Adrenals (g)	**M**	0.0576	0.0596	0.0696	0.0729*	0.0893**
Adrenals (%)		0.012	0.014	0.017*	0.021**	0.028**
Adrenals (g/100 g brain)		2.82	2.94	3.45	3.61*	4.63**
Adrenals (g)	**F**	0.0663	0.0759	0.0705	0.0740	0.0698
Adrenals (%)		0.024	0.031**	0.029	0.031**	0.032**
Adrenals (g/100 g brain)		3.40	3.85	3.59	3.86	3.68
Adrenals: enlarged (#)	**M**	-	-	-	-	1
Epididymides: small (#)		-	-	-	-	3
Coagulating gland: small (pale) (#)		-	-	-	3	9 (1)
Prostate: small (#)		-	-	-	1	9
Coagulating gland: atrophy (#)		-	-	-	-	8
Seminal vesicles: small (#)		-	-	-	3	9
Kidney: mineralisation (#)		1	3	3	NA	9
Prostate: atrophy (#)		-	-	-	-	7
Seminal vesicles: reduced secretion (#)		-	-	-	2	9
Epididymis (g)	**M**	1.40	1.27	1.21**	1.04**	0.78**
Epididymis (%)		0.282	0.292	0.296	0.300	0.243
Epididymis (g/100 g brain)		68.8	62.5	59.8*	51.2*	40.6*
Prostate (g)		1.15	0.97	0.79**	0.53**	0.25**
Prostate (%)		0.233	0.224	0.192	0.153**	0.079**
Prostate (g/100 g brain)		56.6	47.8	39.0**	26.1**	13.3**
Seminal vesicles (g)		2.06	1.83	1.57**	0.97**	0.36**
Seminal vesicles (%)		0.416	0.422	0.385	0.282**	0.109**
Seminal vesicles (g/100 g brain)		101.8	90.5	78.0**	48.1**	18.8**
Testes (g)		3.37	3.27	3.42	3.19	2.47**
Testes (%)		0.678	0.754	0.841**	0.924**	0.771
Testes (g/100 g brain)		165.3	161.6	170.0	157.9	128.4**
Ovaries (g)	**F**	0.1289	0.1252	0.1021**	0.0947**	0.0772**
Ovaries (%)		0.047	0.050	0.042	0.040	0.035**
Ovaries (g/100 g brain)		6.62	6.34	5.49**	4.94**	4.06**

**significantly different to controls ($p<0.05$); **$p<0.01$. # = number of animals. NA= Not assessed.*

v. 90-day rat dietary toxicity study [Vogin, 1970: Food and Drug Research Laboratories]

Groups of FDRL rats (20/sex) were administered TPP in the diet at initial concentrations of 0 (control), 0.05, 0.2 and 0.4%, adjusted to be equivalent to intakes of 0, 25, 100 and 200 mg/kg bw/d. A summary of relevant findings is shown in Table 32.

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/d in males and females.

Findings of relevance to reproductive toxicity

Mean absolute and relative testes weights were reduced in males dosed at 200 mg/kg bw/d with testicular hypospermia observed in six out of 20 animals. Additionally, liver weights were increased among either sex dosed at 200 mg/kg bw/d. No additional histopathological effects were noted in this study.

Summary: This study adds further credibility to the argumentation that effects of TPP on reproductive parameters are secondary to generalized systemic toxicity.

A NOAEL of 100 mg/kg bw/d was assigned for general toxicity and effects on the male reproductive tract.

Table 32: 90-day rat study: summary of findings

Shading illustrates the dose of TPP that resulted in evidence of both systemic toxicity and effects on vital and reproduction organs.

Observation	Sex	Dose level (mg/kg bw/d)			
		0	**25**	**100**	**200**
Terminal bodyweight (g)	**M**	381	388	360	311
	F	229	243	218	204
Weight gain	**M**		-	-	↓
	F		-	-	↓
Food efficiency	**M**	-	-	-	↓
	F		-	-	↓
Liver weight (g)	**M**	14.39	14.88	14.08	13.52
	F	8.26	8.84	8.47	8.38
Liver weight (%)	**M**	3.78	3.84	3.89	4.36***
	F	3.61	3.64	3.89	4.11***
Testes weight (g)	**M**	3.15	3.40	2.99	2.01
Testes weight (%)		0.83	0.88	0.83	0.64*
Hypospermia (#)		1/20	0/20	2/20	6/20

**significantly different to controls ($p<0.05$). # = number of animals*

vi. 90-day dog dietary toxicity study [Vogin, 1970: Food and Drug Research Laboratories]

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/d respectively.

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 organ weights and histopathology did not reveal any effects of treatment. 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.

Summary: This well-conducted 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 the human situation.

A NOAEL of >143 mg/kg bw/d was assigned for general systemic toxicity and effects on the male reproductive tract.

b) Reproductive toxicity studies

i. One-generation reproductive gavage rat toxicity study (Knapp *et al*, 2005; WIL Research Laboratories, WIL-186033)

Groups of CD rats (30/sex) were administered TPP by oral bolus gavage (in corn oil) at dose levels of 0 (vehicle control), 5, 25 or 125 mg/kg bw/d for 10 weeks prior to mating, throughout the mating period and throughout the gestation and lactation of the resulting F_1 offspring. Parental (F_0) animals were terminated on weaning of the F_1 litters at Day 21 *post partum*. Litters were standardised on Day 4 *post partum*. Pups were sacrificed at weaning or were selected for the assessment of developmental landmarks. A summary of relevant findings is shown in Tables 33-36 inclusive.

There were no treatment-related deaths. Signs of general systemic toxicity among parental (F_0) animals included a) excessive salivation and chromodacryorrhoea observed in either sex at dose levels of ≥25 mg/kg bw/d b) reduced bodyweights, reduced weight gain, reduced food consumption and/or reduced food utilisation efficiency observed in either sex at 125 mg/kg bw/d and in males at 25 mg/kg bw/d, c) non-reproduction organ weight effects at ≥25 mg/kg bw/d. Absolute and relative adrenal weight (compared to bodyweight and/or brain) was significantly elevated and hypertrophy of the adrenal cortex *zona fasciculata* was observed among males dosed at 125 mg/kg bw/d; both changes suggestive of a stress response to general systemic toxicity.

<u>Findings of relevance to reproductive toxicity</u>

Mating behaviour was unaffected by treatment, however an effect on fertility was observed at 125 mg/kg bw/d characterised by a reduced conception index, reduced mean litter size and elevated pup mortality. Mean litter size was significantly lower at 125 mg/kg bw/d and only four females conceived in this group, with a mean 3.3 implantation sites compared to 14.1 in control animals. One of these four females failed to deliver, another only delivered a single pup and the remainder had small litters. Survival of pups in this group was also reduced. These effects were not seen at

lowers doses that did not manifest general systemic toxicity. Mean oestrous cycle length was slightly longer in females at 25 and 125 mg/kg bw/d; however, many of the females with a long cycle also had one or more cycles of normal length, diminishing the importance of this observation. At 125 mg/kg bw/d, persistent oestrus was noted for 6/30 females and persistent dioestrus for 16/30 females (including 2 females also showing persistent oestrus). Persistent dioestrus was noted for four females at 25 mg/kg bw/d, compared with 2 females each in controls and at 5 mg/kg bw/d.

Weight gain by pups was significantly reduced at ≥25 mg/kg bw/d from Day 4 *post-partum*, resulting in significantly lower mean bodyweights from Day 14. Mean weights of the small number of surviving pups at 125 mg/kg bw/d were also approximately 50% lower than controls. Sexual maturation of male pups, as measured by balano-preputial separation, was slightly delayed at 25 mg/kg bw/d, but this finding was considered not to be toxicologically significant because it was not associated with a higher mean bodyweight and the time (day) at which maturation occurred was within the normal range for rats of this strain and age. There was no treatment-related effect on the sexual maturation of female pups at this dose level, as measured by vaginal patency. Similar findings were evident among the small numbers of surviving male and female offspring available for assessment at the highest dose level but there were too few survivors to conclude whether a treatment-related effect could be established. Necropsy of pups did not reveal any adverse developmental effects resulting from treatment.

Table 33: One-generation study: findings in parental animals

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

Observation	Sex	Dose level (mg/kg bw/d)			
		0	**5**	**25**	**125**
Signs of toxicity	**M**	-	-	✔	✔
	F	-	-	✔	✔
Pre-mating bodyweight (g)	**M**	530.3	531.1	505.9	421.2**
	F	287.1	281.4	284.2	259.3**
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 (d)	**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 (d)	**F**	4.4	4.6	4.9	5.2
Persistent oestrus (#)	**F**	0	0	0	6
Persistent dioestrus (#)	**F**	2	2	4	16
Gestation length (d)	**F**	21.9	21.7	21.7	22.3

**significantly different to control (P<0.05); **p<0.01.*

Table 34: One-generation study: findings in offspring

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

Observations	Time point	Sex	Dose level (mg/kg bw/d)			
			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
Weight gain (g)	Day 1-4	M	2.5	2.8	2.4	2.9
		F	2.4	2.6	2.3	3.1
	Day 4-7	M	6.2	6.3	5.1*	3.3
		F	5.6	6.0	4.5*	5.8
	Day 7-14	M	17.2	17.4	15.1**	8.4
		F	16.6	17.0	14.5**	12.5
	Day 14-21	M	19.5	19.5	17.6*	12.0
		F	18.2	18.5	16.8*	16.7
Balano-preputial separation (d)		M	43.2	42.9	44.6*	47.5
Balano-preputial separation (g)			230.1	226.0	229.1	205.7
Vaginal patency (d)		F	33.0	32.8	33.5	32.5
Vaginal patency (g)			115.1	116.0	110.2	110.6

**significantly different to control (P<0.05); **p<0.01. # = number of animals*

Absolute and relative (to bodyweight and/or brain) weights of the cauda epididymides, epididymides, prostate, seminal vesicle and testes were significantly lower in males at ≥25 mg/kg bw/d. Weights of the epididymides and prostate were also significantly lower at the highest dose level only. Seminal vesicle weight was also reduced at the lowest dose level tested, 5 mg/kg bw/d, but mean absolute weight was within the laboratory's historical control range and in isolation this finding was attributed to an unusually high concurrent control value and was considered not to be treatment-related. Absolute and relative (to bodyweight and/or brain) weights of the ovaries were

significantly reduced at 25 and 125 mg/kg bw/d, and uterus weight (absolute and relative to bodyweight or brain weight) was increased at 125 mg/kg bw/d.

Table 35: One-generation study: organ weights (parental animals)

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

Organ/tissue	Weight	Dose level (mg/kg bw/d)			
		0	5	25	125
Cauda epididymis left	(g)	0.3489	0.3406	0.3105**	0.2538**
	(%)	0.055	0.052	0.054	0.053
	(g/100 g brain)	16.3	15.8	14.4**	11.8**
Cauda epididymis right	(g)	0.3414	0.3386	0.3110	0.2617**
	(%)	0.053	0.052	0.054	0.055
	(g/100 g brain)	15.9	15.7	14.4*	12.2**
Epididymis left	(g)	0.75	0.74	0.72	0.62**
	(%)	0.117	0.114	0.125	0.132**
	(g/100 g brain)	34.8	34.5	33.2	29.1**
Epididymis right	(g)	0.74	0.75	0.71	0.61**
	(%)	0.116	0.116	0.123	0.129**
	(g/100 g brain)	34.6	35.1	32.8	28.4**
Prostate	(g)	1.04	1.01	10.3	0.67**
	(%)	0.164	0.156	0.180	0.142
	(g/100 g brain)	48.4	47.2	47.9	31.2**
Seminal vesicle	(g)	2.49	2.20**	2.10**	1.39**
	(%)	0.388	0.338**	0.365	0.291**
	(g/100 g brain)	115.6	102.3**	96.8**	64.5**
Testis left	(g)	1.84	1.81	1.82	1.78
	(%)	0.288	0.279	0.317	0.377**
	(g/100 g brain)	85.9	84.4	84.1	83.1
Testis right	(g)	1.84	1.82	1.81	1.71*
	(%)	0.287	0.279	0.316**	0.361**
	(g/100 g brain)	85.5	84.5	84.0	79.7*
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**
Uterus	(g)	0.56	0.59	0.56	0.65
	(%)	0.159	0.175	0.166	0.228**
	(g/100 g brain)	28.9	29.9	29.1	33.9

**significantly different to control (P<0.05); **p<0.01.*

Gross necropsy of parental animals did not reveal any treatment-related effects. Histopathology showed reduced prostate secretion in all groups of treated males, reduced coagulating gland secretion in males dosed at ≥25 mg/kg bw/d and reduced seminal vesicle secretion in males dosed at 125 mg/kg bw/d. It should be noted, however, that the control incidences for reduced secretion in

the prostate and coagulating gland were much higher than seen in other studies (such as the 2-generation study). This questions the robustness of these histopathological examinations. In females, treatment-related effects were apparent at 125 mg/kg bw/d and consisted of increased incidences of oestrus, ovarian (follicular and/or luteal) cysts and endometrial gland cysts. Sperm analysis revealed a significantly reduced epididymal sperm concentration in males at 125 mg/kg bw/d. Mean testicular sperm numbers and sperm production rates, motility, progressive motility and morphology were unaffected by treatment.

Table 36: One-generation study: histopathology (parental animals)

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

Observation	Sex	Dose level (mg/kg bw/d)			
		0	5	25	125
Prostate: reduced secretion (#)	M	6/30	13/29	20/28*	18/30*
Coagulating gland: reduced secretion (#)		9/30	12/29	20/27*	26/30*
Seminal vesicles: reduced secretion (#)		-	-	-	6/30
Kidney: mineralisation (#)		-	1/29	8/28*	21/30*
Epididymis: sperm concentration (10^6/g)		365.2	342.5	316.7	303.2**
Ovary: reduced *corpora lutea*(#)	F	4/30	3/30	4/29	18/30*
Ovary: cysts (#)		4/30	8/30	7/29	15/30*
Kidney: mineralisation (#)		1/30	-	2/29	7/30
Uterus: endometrial cysts (#)		1/30	-	-	8/30*
Vagina: oestrus (#)		3/30	-	3/29	16/30

**significantly different to control (P<0.05); **p<0.01. # = number of animals*

Summary of study findings

The results of this study demonstrate a marked effect on sexual function and fertility at the highest dose level of 125 mg/kg bw/d, with a lesser effect evident at 25 mg/kg bw/d. The effects on fertility were associated with relatively marked effects on bodyweight however, with mean pre-mating bodyweights among males at this dose level reduced to 79% of controls and mean terminal bodyweight at 72% of controls; additionally, mean pre-mating weight gains were 70% and 79% of controls in males and females respectively. Treatment-related effects on reproductive organ weights were observed in animals of either sex at ≥25 mg/kg bw/d together with accompanying microscopic findings. Sperm analysis revealed a significant reduction in epididymal sperm concentration in males at 125 mg/kg bw/d but not in the testes; other parameters were unaffected by treatment. No clear effect on sexual development was seen in this study; the slight but significant delay in balano-preputial separation seen in male offspring at 25 mg/kg bw/d is considered not to be a convincing effect of treatment because it was not associated with a higher mean bodyweight and the time (day) at which maturation occurred was within the normal range for rats of this strain and age.

In summary, this study demonstrates that TPP adversely affects reproductive parameters at dose levels of ≥25 mg/kg bw/d following bolus administration but findings were confounded due to evidence of general systemic toxicity among parental animals and their offspring, in particular reduced bodyweight gain. No treatment-related adverse effects on reproductive parameters could be determined at dose levels that did not elicit general, systemic toxicity.

ii. Two-generation dietary rat toxicity study (Edwards *et al*, 2010; WIL Research Laboratories. WIL-186053)

Groups of CD rats (30/sex) were administered TPP in the diet at dose levels intended to be equivalent to 0, 1.5, 15 and 75 mg/kg bw/d. Rats were treated for at least 10 days prior to mating, throughout mating, gestation and lactation of the resulting F_1 litters. Selected F_1 offspring were treated in a similar manner through to weaning of the F_{2A} litters. To clarify the results from the first mating, the F_1 parental animals were mated again to produce the F_{2B} litters, a minimum of 26 days following weaning of the F_{2A} litters. A summary of relevant findings is shown in Tables 37 through 46.

F_0 parental animals

There were no treatment-related deaths among parental animals. Clinical signs of toxicity were evidenced by decreased defecation in females at 75 mg/kg bw/d and an adverse effect on bodyweights among animals of either sex at this dose level. Bodyweights, weight gain, food consumption and food utilisation efficiency were reduced for either sex at 75 mg/kg bw/d during the pre-mating period. Bodyweights remained lower for females during gestation and lactation as a consequence of the lower bodyweight at mating although weight gains during gestation were comparable in all groups. An early slight weight loss during lactation was seen in F_0 females at 75 mg/kg bw/d but weight gain during late lactation was significantly higher than controls. Adrenal weight (relative to bodyweight) was significantly elevated in 75 mg/kg bw/d males suggestive of a stress response to general systemic toxicity.

Findings of relevance to reproductive toxicity

Reproductive indices in the F_0 generation were unaffected by treatment at dose levels up to 75 mg/kg bw/d. Fertility indices were slightly lower at 75 mg/kg bw/d but values did not attain statistical significance and were within the laboratory's historical control range. Gestation length was unaffected by treatment.

F_0 males dosed at 75 mg/kg bw/d showed a significantly lower epididymal weight and sperm analysis revealed a significantly reduced sperm concentration among these animals. No treatment-related effects on spermatogenesis were seen at lower dose levels and other parameters including testicular sperm concentration, sperm production rate, motility, progressive motility and morphology were unaffected by treatment. It is especially notable that sperm analysis of F_1 males did not reveal any effects of treatment.

Table 37: Two-generation study: findings in F_0 parental animals

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

Observation		Sex	Dose level (mg/kg bw/d)			
			0	1.5	15	75
Intake (mg/kg bw/d)	**Pre-mating**	**M**	-	1.5	15.5	76.0
		F		1.5	15.2	75.5
	Gestation	**F**	-	1.4	15.3	16.8
	Lactation		-	3.5	37.0	174.2
Signs of toxicity	**Pre-mating**	**M**	-	-	-	✔
		F	-	-	-	✔
Bodyweight (g)	**Pre-mating**	**M**	543	548	536	449**
		F	293	290	294	256**
	Gestation Day 20	**F**	413	419	414	369**
	Lactation Day 21		344	346	346	326**
Weight gain g)	**Pre-mating**	**M**	300	305	293	206**
		F	126	123	117	99**
	Gestation Day 20	**F**	125	130	130	120
	Lactation Day 21		25	28	29	43**
Oestrus cycle (d)		**F**	4.3	4.3	4.5	5.4**
Abnormal oestrus (#)			3	2	3	9
Persistent dioestrus (#)			6	3	4	13
Mating index (%)		**M**	93.3	96.7	100.0	93.3
		F	93.3	96.7	100.0	93.3
Fertility index (%)		**M**	93.3	96.7	96.6	86.7
		F	93.3	96.7	96.7	86.7
Copulation index (%)		**M**	100.0	100.0	96.6	92.9
		F	100.0	100.0	96.7	92.9
Epididymal sperm (10^6/g)		**M**	365.2	333.6	357.3	288.5*

**significantly different to control (P<0.05); **p<0.01. # = number of animals*

Absolute and relative (to bodyweight and/or brain) weights of the cauda epididymides, epididymides, prostate, seminal vesicles and testes were significantly lower in males at 75 mg/kg bw/d whereas absolute and relative (to bodyweight and brain) weights of the pituitary were significantly elevated in males of this group. Adrenal weight (relative to bodyweight) was significantly elevated in 75 mg/kg bw/d females whereas absolute and relative (to brain) weights of the organ were not significantly different to controls suggesting this finding was secondary to the bodyweight effects seen at this dose level. Absolute and relative (to bodyweight and brain) weights of the ovaries were significantly reduced in females at 75 mg/kg bw/d; absolute (but not relative) pituitary weight was significantly lower in this group again suggesting this finding was secondary to reduced bodyweight; uterus weights in females at 75 mg/kg bw/d were slightly lower than controls but values did not attain statistical significance.

Gross necropsy of F0 animals did not reveal any treatment-related effects. Histopathology revealed an increased incidence of renal mineralisation in males and reduced numbers of *corpora lutea* in females at 75 mg/kg bw/d. The number of implantation sites was also slightly lower in females at

this dose level. No treatment-related effect was apparent on the numbers of ovary primordial follicles. Oestrus cycle length was increased in females at 75 mg/kg bw/d and the number of rats with abnormal cycles was increased at this dose level.

Table 38: Two-generation study: organ weights (F_0 parental animals)

Shading illustrates the dose of TPP that resulted in evidence of both systemic toxicity and effects on reproduction organ weights.

Sex	Organ/tissue	Weight	Dose level (mg/kg bw/d)			
			0	1.5	15	75
M	Adrenals	(g)	0.0583	0.0593	0.0600	0.0607
		(%)	0.009	0.010	0.010	0.012**
		(g/100g brain)	2.68	2.77	2.79	2.83
	Cauda epididymis left	(g)	0.3666	0.3339	0.3755	0.2747**
		(%)	0.060	0.054	0.062	0.055
		(g/100 g brain)	16.9	15.5	17.5	12.8**
	Cauda epididymis right	(g)	0.3671	0.3529	0.3686	0.2838**
		(%)	0.060	0.057	0.061	0.057
		(g/100 g brain)	16.9	16.5	17.1	13.2**
	Epididymis left	(g)	0.75	0.72	0.76	0.63**
		(%)	0.122	0.115	0.125	0.126
		(g/100 g brain)	34.5	33.4	35.3	29.2**
	Epididymis right	(g)	0.79	0.76	0.79	0.68**
		(%)	0.128	0.123	0.130	0.135
		(g/100 g brain)	36.1	35.5	36.6	31.5**
	Pituitary	(g)	0.0166	0.0163	0.0165	0.0196**
		(%)	0.003	0.003	0.0063	0.004*
		(g/100 g brain)	0.765	0.758	0.766	0.913**
	Prostate	(g)	1.13	1.09	1.09	0.88**
		(%)	0.184	0.177	0.179	0.176
		(g/100 g brain)	52.0	51.0	50.6	41.0**
	Seminal vesicles	(g)	2.48	2.22**	2.31	1.74**
		(%)	0.404	0.359**	0.379	0.346**
		(g/100 g brain)	114.1	103.8*	107.5	81.0**
	Testis left	(g)	1.79	1.69	1.75	1.62*
		(%)	0.291	0.273	0.287	0.326**
		(g/100 g brain)	82.1	78.9	81.2	75.7
	Testis right	(g)	1.78	1.74	1.70	1.66
		(%)	0.289	0.281	0.279	0.333**
		(g/100 g brain)	81.6	81.3	78.9	77.3
F	Adrenals	(g)	0.0751	0.0752	0.0762	0.0745
		(%)	0.023	0.023	0.024	0.026**
		(g/100g brain)	3.68	3.74	3.78	3.81
	Ovaries	(g)	0.1202	0.1210	0.1142	0.0846**
		(%)	0.037	0.038	0.036	0.029**

Sex	Organ/tissue	Weight	Dose level (mg/kg bw/d)			
			0	1.5	15	75
		(g/100 g brain)	5.89	6.00	5.66	4.33**
	Pituitary	(g)	0.0210	0.0190	0.0184	0.0172**
		(%)	0.006	0.006	0.006	0.006
		(g/100 g brain)	0.985	0.942	0.909	0.882
	Uterus	(g)	0.74	0.72	0.90	0.66
		(%)	0.232	0.224	0.281	0.232
		(g/100 g brain)	36.5	35.6	44.8	33.7

**significantly different to control (P<0.05); **p<0.01.*

Table 39: Two-generation study: histopathology (F0 parental animals)

Shading illustrates the dose of TPP that resulted in evidence of both systemic toxicity and effects on reproduction organ weights.

Observation	Sex	Dose level (mg/kg bw/d)			
		0	1.5	15	75
Prostate: reduced secretion (#)	M	0/30	-	-	0/30
Coagulating gland: reduced secretion (#)		0/30	-	-	0/30
Seminal vesicles: reduced secretion (#)		0/30	-	-	0/30
Kidney: mineralisation (#)		1/30	0/29	4/29	26/30*
Epididymis: sperm concentration (10^6/g)		365.2	333.6	357.3	288.5*
Ovary: decreased *corpora lutea*(#)	F	1/30	0/27	0/30	6/28*
Ovary: cysts (#)		6/30	6/27	13/30	10/30
Kidney: mineralisation (#)		10/30	-	-	10/29
Uterus: endometrial cysts (#)		0/30	-	-	0/29
Vagina: oestrus (#)		-	-	-	-

**significantly different to control (P<0.05); **p<0.01.* Stage of oestrous cycle not reported in this study

Findings in F_1 offspring

F_1 litter size and post-natal survival were not affected by treatment. Litter size at 75 mg/kg bw/d was slightly lower than controls, secondary to the number of implantation sites in this group. Early post-natal survival at 75 mg/kg bw/d was marginally lower than controls but this finding was considered not to be treatment-related because it did not attain statistical significance, values were within the laboratory's historical control range, and was attributable to findings in two litters. Mean F_1 pup weights were significantly lower at 15 and 75 mg/kg bw/d from Day 7. Weight gains by F_1 pups at 75 mg/kg bw/d were significantly lower than controls from Day 1; from Day 4 at 15 mg/kg bw/d and from Day 14 in females only at 1.5 mg/kg bw/d the lowest dose level tested. However, these animals showed the same weight gain as those dosed at 15 mg/k bw/d and in the absence of a convincing dose-response relationship the toxicological significance of this finding is dubious. There was no convincing direct effect on sexual maturation; the mean age at which balano-preputial separation occurred was significantly increased in F_1 male offspring dosed at 75 mg/kg bw/d but this finding was associated with a significantly lower bodyweight amongst these animals and was therefore considered not to represent a direct effect of treatment. It has been shown in other studies that when growth is retarded, preputial separation and vaginal patency are delayed, but that the weight at preputial separation and vaginal patency was similar to that in controls (Engelbregt *et al*, 2000; Kennedy & Mitra, 1963). Among females dosed at 75 mg/kg bw/d, vaginal patency was

attained by F_1 pups at a significantly earlier age and was also associated with a significantly lower mean bodyweight. Anogenital distance was unaffected by treatment. Gross necropsy of decedents and non-selected pups did not reveal any effects of treatment. Effects on mean brain, thymus and spleen weights were observed in non-selected offspring of the treated groups but were considered to be secondary to bodyweight effects and not to represent a direct effect of treatment.

Table 40: Two generation study: findings in F_1 offspring

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

Observation	Time point	Sex	Dose level (mg/kg bw/d)			
			0	1.5	15	75
Mean litter size (#)	**Day 0**	**M/F**	13.4	13.0	13.2	12.6
Viability (%)	**Day 0**	**M/F**	99.3	99.8	98.1	96.8
	Day 0-1	**M/F**	99.1	98.9	98.7	91.8
	Day 1-4	**M/F**	98.4	99.2	99.3	98.2
	Day 4-21	**M/F**	99.5	96.2	96.9	94.8
Pup weight(g)	**Day 1**	**M**	7.5	7.5	7.3	7.2
		F	7.0	7.0	6.9	6.8
	Day 4	**M**	10.4	10.2	10.0	9.6
		F	9.8	9.6	9.6	9.0
	Day 7	**M**	16.5	16.1	15.1*	14.3**
		F	15.7	15.3	14.4**	13.5**
	Day 14	**M**	30.1	30.1	28.6	22.9**
		F	29.2	29.1	27.3	21.8**
	Day 21	**M**	50.7	49.1	47.0	36.4**
		F	49.3	46.8	45.0**	34.4**
Weight gain (g)	**Day 1-4**	**M**	2.9	2.7	2.7	2.4
		F	2.8	2.6	2.7	2.2*
	Day 4-7	**M**	6.2	5.9	5.1**	4.7**
		F	6.0	5.7	4.9**	4.4**
	Day 7-14	**M**	13.6	14.0	13.4	8.6**
		F	13.5	13.7	12.9	8.3**
	Day 14-21	**M**	20.6	19.0	18.4*	13.1**
		F	20.1	17.7**	17.7**	12.6**
Balano-preputial separation (d)		**M**	45.1	45.5	45.8	47.1*
Balano-preputial separation (g)			246.2	247.4	237.6	226.4**
Vaginal patency (d)		**F**	32.4	32.2	32.4	27.4**
Vaginal patency (g)			112.0	110.3	105.0	60.8**

**significantly different to control (P<0.05); **p<0.01. # = number of animals*

Findings in F_1 parental animals

Five selected F_1 animals at 75 mg/kg bw/d were found dead or killed *in extremis* shortly after weaning; deaths occurred in animals with low bodyweights and were considered to be treatment-related. No signs of toxicity were observed however. Mean bodyweights of males and females at 75 mg/kg bw/d were significantly lower than controls throughout the pre-mating period (F_{2A}

generation) as a consequence of significantly reduced weight gain. Mean bodyweights of females in this group remained significantly lower throughout gestation and lactation of the F_{2A} generation; weight gain during gestation was comparable to controls and increased weight gain was observed during lactation. Mean bodyweights of females in this group remained significantly lower than controls at the second mating (F_{2B} generation) as a consequence of slight weight loss and were also significantly lower throughout gestation, but were comparable to controls at the end of the lactation period. Weight gain during gestation was comparable to controls and again increased weight gain was observed during lactation.

Reproduction indices for the first mating were lower than controls at 1.5 and 15 mg/kg bw/d but because values at 75 mg/kg bw/d were comparable to controls and a clear dose-response relationship could not be demonstrated a second mating performed (the same animals were paired) to clarify the significance of these findings. Following the second mating, reproduction indices in animals at 1.5 and 15 mg/kg bw/d were comparable to controls. Fertility and copulation indices at 75 mg/kg bw/d were lower than controls but values in all groups are low as a consequence of the age of animals at the second mating and data for this second mating phase (2^{nd} mate) cannot be considered to be robust. Gestation length was unaffected by treatment for either mating.

In contrast with findings in F_0 males, sperm analysis did not reveal any adverse effects of treatment. The increased testis sperm concentration and increased sperm production rate observed in males at 75 mg/kg bw/d were within the laboratory's historical control range and were therefore considered to be due to unusually low concurrent control values.

Mean oestrus cycle length was significantly increased in females at 75 mg/kg bw/d; many females had cycles of variable length, some being normal and some more prolonged. Prolonged dioestrus was also observed in a large proportion of animals in this group.

Table 40: Two generation study: findings in F_1 parental animals

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

Observation		Sex	Dose level (mg/kg bw/d)			
			0	1.5	15	75
Pre-mating bodyweight (g)	1st mate	M	573	579	545	444**
		F	319	313	309	279**
	2nd mate	M	711	740	679	527**
		F	377	367	366	306**
Gestation Day 20 bodyweight (g)	1st mate	F	447	436	436	388**
	2nd mate		483	483	477	423**
Lactation Day 21 bodyweight (g)	1st mate	F	367	372	359	337**
	2nd mate		378	383	382	359
Pre-mating weight gain (g)	1st mate	M	380	395	361	283**
		F	164	167	159	145**
	2nd mate	M	518	556	494	366**
		F	15	18	13	-1*
Gestation weight gain (g)	1st mate	F	130	121	130	122
	2nd mate		122	120	124	111
Lactation weight gain (g)	1st mate	F	14	26*	16	41**
	2nd mate		-11	-3	0	22**
Oestrus cycle (d)		F	4.3	4.2	4.6	6.5**
Abnormal oestrus (#)			5	3	11	22
Prolonged dioestrus (#)			5	3	11	21
Mating index (%)	1st mate	M	93.1	100	95.7	96.3
		F	93.1	100	95.7	96.3
Fertility index (%)		M	92.8	76.7	71.4	85.2
		F	92.8	76.7	71.4	85.2
Copulation index (%)		M	88.9	76.7	83.3	88.5
		F	88.9	76.7	83.3	88.5
Mating index (%)	2nd mate	M	75.9	83.3	77.8	92.6
		F	75.9	83.3	75.0	92.6
Fertility index (%)		M	65.5	80.0	70.4	55.6
		F	65.5	80.0	70.4	55.6
Copulation index (%)		M	86.4	96.0	90.5	60.0
		F	86.4	96.0	90.5	60.0
Testis sperm (10^6/g)	F1	M	53.6	59.6	65.9*	65.1*
Sperm production (10^6/g/d)	F1	M	8.8	9.8	10.8*	10.7*

**significantly different to control (P<0.05); **p<0.01. # = number of animals*

Mean relative (to bodyweight) adrenal weight was slightly (but significantly) elevated in animals of either sex dosed at at 75 mg/kg bw/d with absolute weights also elevated in females at this dose level, changes suggestive of a stress response. Mean absolute weights of the cauda epididymides, epididymides, prostate, seminal vesicles and testes were significantly reduced in males at 75 mg/kg bw/d; weights of these organs relative to bodyweight were generally significantly higher than controls as a consequence of the lower bodyweights in this group but organ weights expressed relative to brain weight are lower, significantly for the seminal vesicles suggesting a direct effect not due to reduced bodyweight. Mean absolute and relative (to bodyweight and brain) pituitary weights were significantly elevated in males at 75 mg/kg bw/d. Mean ovary weights were significantly lower in females at 75 mg/kg bw/d. Uterus weight, relative to bodyweight, was increased but there was no evidence of a treatment-related effect on absolute weight or uterine weight relative to brain weight again suggesting this finding was secondary to the bodyweight effect evident at this dose level.

Table 41: Two-generation study: organ weights (F_1 parental animals)

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

Sex	Organ/tissue	Weight	**Dose level** (mg/kg bw/d)			
			0	**1.5**	**15**	**75**
M	**Adrenals**	(g)	0.0595	0.0600	0.0605	0.0565
		(%)	0.008	0.007	0.008	0.010**
		(g/100 g brain)	2.70	2.79	2.82	2.71
	Cauda epididymis left	(g)	0.3028	0.3362	0.3391*	0.2740
		(%)	0.039	0.042	0.046**	0.049**
		(g/100 g brain)	13.8	15.7*	15.8*	13.1
	Cauda epididymis right	(g)	0.3349	0.3588	0.3372	0.2879**
		(%)	0.043	0.045	0.045	0.052**
		(g/100 g brain)	15.3	16.7	15.7	13.8
	Epididymis left	(g)	0.67	0.73	0.75*	0.65
		(%)	0.085	0.092	0.101**	0.115**
		(g/100 g brain)	30.5	34.2**	35.1**	31.0
	Epididymis right	(g)	0.76	0.80	0.77	0.68**
		(%)	0.097	0.100	0.104	0.121**
		(g/100 g brain)	34.7	37.1	36.1	32.6
	Pituitary	(g)	0.0165	0.0164	0.0173	0.0191**
		(%)	0.002	0.002	0.002	0.003**
		(g/100 g brain)	0.747	0.762	0.807	0.913**
	Prostate	(g)	1.06	1.07	1.06	0.92*
		(%)	0.136	0.135	0.141	0.164**
		(g/100 g brain)	48.1	49.9	49.2	44.0
	Seminal vesicles	(g)	2.19	2.26	2.20	1.81**
		(%)	0.279	0.284	0.296	0.323**
		(g/100 g brain)	99.3	104.9	102.6	86.6**
	Testis left	(g)	1.87	1.94	1.94	1.74
		(%)	0.237	0.243	0.260	0.311**

Sex	Organ/tissue	Weight	Dose level (mg/kg bw/d)			
			0	1.5	15	75
		(g/100 g brain)	84.8	90.4	90.4	83.5
	Testis right	(g)	1.93	1.96	1.88	1.72**
		(%)	0.245	0.245	0.252	0.306**
		(g/100 g brain)	87.6	91.3	87.6	82.2
F	Adrenals	(g)	0.0711	0.0724	0.0605	0.0819**
		(%)	0.018	0.019	0.019	0.026**
		(g/100 g brain)	3.60	3.67	3.71	4.33**
	Ovaries	(g)	0.1051	0.0993	0.1027	0.0651**
		(%)	0.026	0.026	0.027	0.021*
		(g/100 g brain)	5.32	5.04	5.26	3.42**
	Pituitary	(g)	0.022	0.020	0.031	0.021
		(%)	0.005	0.005	0.009	0.007
		(g/100 g brain)	1.11	1.02	1.58	1.13
	Uterus	(g)	0.74	0.84	0.77	0.74
		(%)	0.183	0.219	0.199	0.239**
		(g/100 g brain)	37.5	42.6	39.2	39.2

**significantly different to control (P<0.05); **p<0.01.*

Table 42: Two-generation study: histopathology (F1 parental animals)

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

Observation	Sex	Dose level (mg/kg bw/d)			
		0	1.5	15	75
Prostate: reduced secretion (#)	M	0/30	-	-	0/28
Coagulating gland: reduced secretion (#)		0/30	-	-	0/28
Seminal vesicles: reduced secretion (#)		0/30	-	-	0/28
Kidney: mineralisation (#)		3/30	4/30	10/29*	27/28*
Epididymis: sperm concentration (10^6/g)		310.1	339.4	350.2	320.5
Ovary: decreased *corpora lutea*(#)	F	6/28	2/28	3/30	16/26*
Ovary: cysts (#)		17/28	11/28	14/30	9/26
Kidney: mineralisation (#)		18/28	-	-	23/26
Uterus: endometrial cysts (#)		0/28	-	-	0/26
Vagina: oestrus (#)		-	-	-	-

*significantly different to control (P<0.05); **p<0.01. Stage of oestrous cycle not reported in this study

Gross necropsy did not reveal any effects of treatment. Microscopic evaluation of the 75 mg/kg bw/d rats of either sex dying shortly after weaning revealed immature reproductive tract tissues. These findings were considered to be indicative of delayed development as a consequence of low bodyweight and were not evident among F_1_males at scheduled termination. At scheduled termination, histopathology revealed renal tubular mineralisation in male rats dosed at 15 and 75 mg/kg bw/d and reduced number of corpora lutea and implantation sites among females dosed at 75 mg/kg bw/d.

Findings in F_{2A} offspring

Mean litter size at 75 mg/kg bw/d was slightly lower than controls. As with F_1 offspring early post-natal survival at was slightly reduced at 75 mg/kg bw/d as a consequence of mortality in two litters (in one litter of 9 pups, 4 died on Day 0 and another 4 died on Day 1; in another litter of 12 pups, 8 died on Day 0-1, and there was total litter loss by Day 7). Except for these two litters survival of pups after Day 4 *post-partum* was comparable in all groups. Anogenital distance in male and female pups was comparable in all dose groups. Mean pup weights at 75 mg/kg bw/d were significantly lower than controls from birth and throughout lactation; weight gains in this group were also significantly lower than controls from Day 4 *post-partum*. Gross necropsy did not reveal any effects of treatment and organ weights were not directly affected by treatment.

Table 43: Two generation study: findings in F_{2A} offspring

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

Observation	Time point	Sex	Dose level (mg/kg bw/d)			
			0	1.5	15	75
Litter size (#)	**Day 0**	**M/F**	13.4	13.0	13.2	12.6
Viability (%)	**Day 0**	**M/F**	99.3	99.8	98.1	96.8
	Day 0-1	**M/F**	99.1	98.9	98.7	91.8
	Day 1-4	**M/F**	98.4	99.2	99.3	98.2
	Day 4-21	**M/F**	99.5	96.2	96.9	94.8
Pup weight(g)	**Day 1**	**M**	7.4	7.4	7.1	6.7*
		F	7.0	6.9	6.7	6.3**
	Day 4	**M**	10.5	10.8	10.5	9.8
		F	9.9	10.2	9.6	9.1
	Day 7	**M**	16.8	17.4	16.8	15.4
		F	15.9	16.3	15.3	14.2*
	Day 14	**M**	33.9	34.9	33.7	29.0**
		F	32.5	33.7	31.5	27.9**
	Day 21	**M**	51.9	52.6	52.7	40.9**
		F	49.6	50.5	48.9	39.4**
Weight gain (g)	**Day 1-4**	**M**	3.1	3.4	3.4	3.0
		F	2.9	3.2	2.9	2.8
	Day 4-7	**M**	6.3	6.5	6.3	5.6*
		F	6.0	6.1	5.6	5.1*
	Day 7-14	**M**	17.1	17.5	16.9	13.5**
		F	16.6	17.4	16.2	13.4**
	Day 14-21	**M**	18.0	17.6	19.0	12.0**
		F	17.0	16.9	17.4	11.5**
Anogenital distance (mm)		**M**	4.48	4.54	4.41	4.39
		F	2.51	2.56	2.50	2.44
Anogenital distance (relative to bodyweight)		**M**	2.30	2.33	2.29	2.33
		F	1.32	1.34	1.33	1.32

**significantly different to control (P<0.05); **p<0.01. # = number of animals*

Findings in F_{2B} offspring

Poor reproductive performance was observed in all groups in this generation and this can be attributed to the advanced age of the animals. Findings in F2B offspring confirmed those observed among F_{2A} offspring except that mean litter size was significantly reduced at 75 mg/kg bw/d in F_{2B} offspring. Early post-natal survival of offspring was also reduced in this group, although this was largely due to the total loss of one litter of 11 pups by Day 2. Survival of pups after Day 4 *post-partum* was comparable in all groups. Mean pup weights at birth were not significantly affected by treatment but pup weights at 75 mg/kg bw/d were significantly lower than controls from Day 14 *post-partum* as a consequence of reduced weight gain. Gross necropsy did not reveal any effects of treatment and organ weights were not directly affected by treatment.

Table 44: Two generation study: findings in F_{2B} offspring

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

Observation	Time point	Sex	Dose level (mg/kg bw/d)			
			0	1.5	15	75
Litter size (#)	**Day 0**	**M/F**	13.4	13.1	13.3	10.1*
Viability (%)	**Day 0**	**M/F**	99.3	93.9	97.8	94.9
	Day 0-1	**M/F**	99.6	98.5	97.8	91.4
	Day 1-4	**M/F**	98.5	98.1	94.3	90.2
	Day 4-21	**M/F**	96.3	97.0	97.4	96.2
Pup weight(g)	**Day 1**	**M**	7.4	7.4	7.1	7.1
		F	7.0	7.0	6.7	6.8
	Day 4	**M**	10.6	11.0	10.1	10.2
		F	10.0	10.3	9.5	10.1
	Day 7	**M**	16.8	17.4	15.6	15.3
		F	15.8	16.3	14.7	15.2
	Day 14	**M**	33.9	34.9	31.8	28.4**
		F	32.5	33.2	30.7	28.4*
	Day 21	**M**	53.1	54.3	51.2	42.8**
		F	50.0	51.3	48.3	42.1**
Weight gain (g)	**Day 1-4**	**M**	3.2	3.5	3.0	3.0
		F	3.0	3.3	2.8	3.1
	Day 4-7	**M**	6.2	6.4	5.4	5.1*
		F	5.8	6.0	5.2	5.1
	Day 7-14	**M**	17.1	17.5	16.3	13.1**
		F	16.6	16.9	16.1	13.2**
	Day 14-21	**M**	19.2	19.4	19.4	14.4**
		F	17.5	18.1	17.6	13.7**

**significantly different to control (P<0.05); **p<0.01. # =- number of animals*

Summary of study findings

No effects on reproduction were observed in the F_0 generation at the highest dose level examined (75 mg/kg bw/d); this dose level was sufficient to cause systemic toxicity in males and females, including lower mean bodyweights and reduced weight gain. Significantly reduced litter size was observed for the F_{2B} offspring at 75 mg/kg bw/d, however the significance of this finding is unclear due to the absence of a similar finding among F_{2A} offspring and the poor reproductive performance

in all groups in this generation as a consequence of the age of the animals. Slight effects on post-natal survival were observed in the F_{2A} and F_{2B} offspring at 75 mg/kg bw/d, however dietary concentrations were not adjusted during the lactation period, therefore it is plausible that actual dose levels administered during the lactation period were actually significantly higher than the nominal dose level. For example, actual mean high dose levels administered during the lactation period were calculated to be 174 (range: 115-205), 166 (105-187) and 158 (102-188) mg/kg bw/d for the F_1, F_{2A} and F_{2B} litters respectively compared with the nominal dose level of 75 mg/kg bw/d (Table 45) Other adverse effects seen in this study included reduced weights of male reproductive organs, reduced ovary weights and corpora lutea, prolonged oestrus cycles and persistent dioestrus and accelerated female sexual development, and such findings are usually consistent with an oestrogenic effect of the test material. However only mild and inconsistent effects were apparent on sperm parameters at the highest dose level (reduced epididymal sperm count in F_0 males was not confirmed in F_1 males), there was no evidence of an effect on uterus weight and there was no consistent effect on fertility in this study.

Table 45: Calculated actual dosage for F_0, F_1 and F_2 animals compared with nominal dietary dose levels

Stage		Sex	Dose level (mg/kg bw/d)			
			0	1.5	15	75
Intake (mg/kg bw/d)	F0 Pre-mating	M	-	1.5	15.5	76.0
		F		1.5	15.2	75.5
	F0 Gestation	F	-	1.4	15.3	76.8
	F0 Lactation		-	3.5	37.0	174.2
	F1 Pre-mating	M	-	1.6	15.8	78.6
		F	-	1.6	15.5	77.2
	F1 Gestation, 1st mate	F	-	1.4	13.3	72.0
	F1 Lactation, 1st mate		-	3.3	32.5	166.1
	F1 Gestation, 2nd mate	F	-	1.3	14.7	72.8
	F1 Lactation, 2nd mate		-	3.1	32.8	157.7

In summary, this two-generation reproduction toxicity study demonstrates that continuous administration of TPP at dose levels of up to 75 mg/kg bw/d did not elicit a convincing, direct adverse effect on sexual function or fertility in these animals. Study findings were again confounded due to evidence of general systemic toxicity among parental animals and their offspring, in particular reduced bodyweight gain, although to a lesser extent than in the previous one-generation study where signs of toxicity following bolus administration were more severe. Again, no treatment-related adverse effects on reproductive parameters could be determined at dose levels that did not elicit general, systemic toxicity.

4.11.1.2 Human information

No data are available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

The key study for developmental toxicity following oral exposure (Schroeder, 1987) the study was conducted according to OECD Guideline 414 (Prenatal Developmental Toxicity Study) and in accordance with GLP.

Groups of 24 mated female CD rats were administered TPP (in corn oil) by oral bolus gavage at dose levels of 0 (vehicle control), 20, 100 or 300 mg/kg bw/d on Days 6-15 of gestation. An additional group administered 500 mg/kg bw/d was terminated early due to excessive mortality. Dams were sacrificed on Day 20 of gestation and the uterine contents examined. All foetuses were assessed for external malformations; approximately half of the foetuses in each litter were investigated for soft tissue findings by micro dissection and the remaining foetuses assessed for skeletal effects following staining with Alizarin Red S. A summary of relevant findings is shown in Tables 46 and 47.

No deaths occurred in dams administered dose levels of up to 300 mg/kg bw/d. Signs of toxicity were limited to soft stool at the highest dose level together with significantly reduced bodyweight gain and reduced food consumption. Although mean bodyweights of dams at 300 mg/kg bw/d were significantly higher than controls at the start of the study period, markedly reduced weight gain in this group especially during the period when TPP was being administered resulted in significantly lower bodyweights by the end of the study. Bodyweights at 20 and 100 mg/kg bw/d were unaffected by treatment. Gross necropsy of dams did not reveal any treatment-related effects.

Numbers of *corpora lutea* and implantations were comparable in all groups; pre-implantation loss was slightly (but not significantly) higher at 300 mg/kg bw/d. Litter size at 300 mg/kg bw/d was significantly lower than controls, largely as a consequence of increased resorption (including total resorption in one dam) and mean foetal weight was significantly lower at this dose level.

Table 46: Developmental toxicity study: maternal findings

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

Observation	Time point	Dose level (mg/kg bw/d)			
		0	20	100	300
Signs of toxicity		-	-	-	✔
Mated (#)		24	24	24	24
Pregnant (#)		24	24	23	24
Total resorption (#)		-	-	-	1
Litters (#)		24	24	23	23
Bodyweight (g)	**Day 0**	205	207	207	222**
	Day 6	240	239	238	252
	Day 15	289	287	288	271
	Day 20	358	355	357	329*
Weight gain (g)	**Day 6-15**	50	48	50	19**
	Day 15-20	69	68	68	58*
Food consumption (g)	**Day 6-10**	87	81	81	68**
	Day 10-15	90	90	86	77**
Corpora lutea (#)		14.5	13.8	15.1	15.0
Implantations (#)		13.3	13.1	13.7	12.9
Pre-implantation loss		0.077	0.052	0.083	0.132
Litter size (#)		12.5	12.3	13.1	8.9**
Resorptions (#)		0.8	0.8	0.6	4.0*
Resorptions (%)		0.052	0.057	0.044	0.307
Foetal weight (g)		3.76	3.75	3.73	3.47**

**significantly different to control (P<0.05); **p<0.01 # = number of animals*

External findings

The total incidence of external malformations was elevated at 300 mg/kg bw/d with malformations observed in four foetuses between two litters. The three incidences of cleft palate were observed in foetuses from a single litter suggesting a genetic element; however this dam demonstrated marked weight loss and signs of toxicity over the treatment period. One of the foetuses with cleft palate also showed brachydactyly, ectrodactyly and an absent claw. The remaining external malformations (ectrodactyly and short tail) were observed in a single foetus from a different dam. The total incidence of external variations was significantly higher in foetuses at 300 mg/kg bw/d, largely due to incidences of kinked tail and pale foetus.

Visceral findings

The total incidence of visceral malformations and variations was comparable in all groups.

Skeletal findings

The total incidence of skeletal malformations was significantly increased in foetuses at 300 mg/kg bw/d with the incidence of wavy rib (classed as a malformation in the study report but more accurately considered to be a developmental variation) markedly increased in this group. Other findings included curved scapula/scapular spine and abnormally shaped long bones (humerus, ulna, radius and femur) which occurred together in the same foetuses. The total incidence of skeletal variations was also significantly higher in foetuses at 300 mg/kg bw/d, with findings due to reduced/delayed ossification of a number of bones. The total incidence of skeletal variations was

also slightly (but significantly) higher in foetuses at 20 mg/kg bw/d. Analysis of individual findings does not indicate any consistent or dose-related trend in this group however with the incidence of some findings at 20 mg/kg bw/d actually below that of the concurrent control and these findings were considered not to be toxicologically significant.

Table 47: Developmental toxicity study: foetal findings

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

Observation	**Dose level** (mg/kg bw/d)			
	0	**20**	**100**	**300**
External malformations	-	1 (1)	-	4 (2)
Anophthalmia	-	1 (1)	-	-
Ectrodactyly	-	-	-	2 (2)
Short tail	-	-	-	1 (1)
Cleft palate	-	-	-	3 (1)
Brachydactyly	-	-	-	1 (1)
Absent claw	-	-	-	1 (1)
External variations	3 (2)	1 (1)	-	12** (6)
Kinked tail	-	-	-	6 (4)
Pale	-	-	-	5 (2)
Visceral malformations	-	1(1)	1(1)	1(1)
Visceral variations	12 (8)	25 (12)	12 (10)	8 (6)
Skeletal malformations	-	2 (1)	1 (1)	23** (12**)
Palatine processes do not meet	-	-	-	2 (1)
Wavy ribs	-	2 (1)	1 (1)	22 (12)
Scapula / scapular spine curved	-	-	-	10 (6)
Humerus thickened	-	-	-	3 (3)
Humerus curved	-	-	-	3 (1)
Humerus misshapen	-	-	-	1 (1)
Ulna shortened	-	-	-	1 (1)
Ulna curved	-	-	-	3 (2)
Radius shortened	-	-	-	1 (1)
Radius curved	-	-	-	4 (2)
Femur curved	-	-	-	2 (1)
Femur short	-	-	-	1 (1)
Skeletal variations	49.3% (100%)	56.0% (100%)	63.4%* (100%)	74.8%** (100%)

*No. affected foetuses (no. affected litters) *significantly different to control (P<0.05); **p<0.01*

In this rat developmental toxicity study, increased incidences of external and skeletal malformations and variations were observed at the highest dose level of 300 mg/kg bw/d. Marked maternal toxicity was observed in this group and there is a clear association between litters with malformations and individual dams with the most marked bodyweight effects in this study. It is also notable that an additional group of rats administered 500 mg/kg bw/d was terminated early due to excessive mortality. No treatment-related developmental effects were evident at dose levels where systemic toxicity, particularly adverse effects on bodyweight gain, was absent.

4.11.2.2 Human information

No available information.

4.11.3 Other relevant information

No available information.

4.11.4 Summary and discussion of reproductive toxicity

Relevant data are extracted from the repeated dose systemic and reproductive toxicity studies performed with TPP. Data are summarised and compared against the criteria for classification for reproductive toxicity under the CLP Regulation, and published opinions of the Committee for Risk Assessment (RAC) concerning substances classified for reproductive effects. Based on a consideration of all of these factors it is concluded that TPP is most appropriately classified under CLP Regulation as:

4.11.5 Comparison with criteria

a) Fertility effects

i. Comparison with classification criteria for reproductive toxicity (sexual function and fertility)

Criteria for Category 2: Suspected 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, 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.

Key considerations:

- Existing data indicates that TPP affects sexual function and fertility in the rat, but only at doses that also produce general systemic toxicity. At TPP doses that are below the threshold of systemic toxicity, sexual function and fertility are not adversely affected. The data supports the notion that the effects of TPP on sexual function and fertility are secondary consequence of other general toxic effects.

- Repeated dose studies in rats using the oral bolus gavage route of administration resulted in a greater degree of systemic toxicity, with evidence of effects on reproduction function, which was not observed at comparable doses in dietary studies. This suggests that oral bolus gavage is an inappropriate route of administration to study the reproduction toxicity of TPP in rats. It has been observed that even at moderate doses, alkyl phenols administered by the oral bolus gavage method can overload the hepatic detoxification systems resulting in unrealistic levels of substance in the circulation, thus enhancing the toxicity profile. Thus, repeated dose studies using the oral bolus gavage method of administration is considered to be less reliable for studying the effects of TPP on sexual function and fertility.

Criteria for Category 1B: Presumed reproductive toxicant

The classification of a substance in 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 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:

- Existing data indicates that TPP affects sexual function and fertility in the rat, but only at doses that also produce general systemic toxicity. At TPP doses that are below the threshold of systemic toxicity, sexual function and fertility are not adversely affected. The data supports the notion that the effects of TPP on sexual function and fertility are secondary consequence of other toxic effects.

- Effects on the reproductive system of rats were not observed in dogs at equivalent doses. The lack of reproducibility of reproductive effects in an alternative species calls into question the relevance of the rat findings to humans.

Criteria for Category 1A: Known human reproductive toxicant

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

There is no information available which supports a known adverse effect of TPP in humans. Assignment of TPP to this classification category is therefore not appropriate.

Summary: An objective and careful review of the existing data for TPP supports the most appropriate placement in Category 2 for reproductive toxicity (effects on fertility).

ii. Consistency with previous RAC opinions

Among the relevant considerations in the proposed classification is a comparison of the available evidence on TPP in comparison with the evidence and rationale that the RAC used to develop their classification opinion for other substances.

Of the published RAC opinions on harmonised classification and labelling, a number include classification for reproductive toxicity (effects on sexual function and fertility) in either Category 1B or Category 2. A summary of relevant data that RAC indicated had informed their opinions on the assignment of each of these substances into the relevant classification for reproductive toxicity is given in Table 1, and this rationale is compared to the effects observed for TPP.

Table 1: Comparison of TPP hazard profile with RAC opinion on hazard category for substances classified for reproductive effects

Category 1b decisions

Di-n-hexylphthalate [Category 1B; H360f]	**TPP**
Testicular toxicity (intense degeneration or complete atrophy of the seminiferous tubules) was observed in rats and was associated with epididymal oligospermia and	Effects on the reproductive tract in the rat were only apparent at dose levels causing general systemic toxicity or marked bodyweight reductions. Effects on the male

azoospermia. Similar effects are seen in the mouse.	reproductive tract are observed in the rat but not in the dog.
Effects on fertility have not been investigated in rats, however reduced fertility was observed in a continuous breeding study in mice. The effect on fertility was marked and was associated with a severe reduction in testis weight, extensive atrophy of the seminiferous tubules and severe reductions in epididymal; sperm concentration and motility. Effects were observed at all dose levels and in the absence of general toxicity.	No effects on fertility were observed in a definitive two-generation dietary toxicity study, even at doses producing toxicity. Effects on fertility were seen in a bolus dosing one-generation study, but these were associated with general systemic toxicity and marked body weight effects.

2-Ethoxyethanol [Category 1B; H360f]	**TPP**
Data from various species indicate that 2-ethoxyethanol specifically affects the male reproductive tract and is spermatotoxic at low dose levels.	Effects on the male reproductive tract were shown in one species (rat) but not in the other species (dog). Effects on the testes were only apparent at dose levels resulting in evidence of systemic toxicity. Inconsistent effects were observed on sperm count, at dose levels causing relatively marked effects on bodyweight. More minor effects on the male reproductive tract were observed at lower dose levels and were associated with general toxicity (bodyweight effects).
Epidemiology data indicate an association between exposure to 2-ethoxyethanol and impairment of reproduction; mainly a negative influence on sperm count and sperm morphology.	No epidemiological data are available to establish relevance of effects seen in rats to humans

4-tert-butylbenzoic acid [Category 1B; H360f]	**TPP**
Several repeated dose toxicity studies in the rat indicate an effect on the testes (reduced weight, atrophy) and spermatotoxicity (degeneration of the seminiferous tubules, destruction of the germinative epithelium and the loss of late spermatids) at relatively low dose levels.	Effects on reproductive function in all studies were seen at dose levels producing general toxicity. At lower doses, effects on male reproductive tract (accessory organs) were associated with bodyweight reductions, and were relatively mild and of no apparent functional consequence.
In some studies testicular toxicity occurred at dose levels at which bodyweight gain was also significantly affected, however other studies report that testicular toxicity was evident at doses without any sign of general toxicity.	Effects on the testes are associated with marked general toxicity (bodyweight effects); other effects on the male reproductive tract are associated with general toxicity. Inconsistent effects were observed on sperm count, at dose levels causing relatively marked effects on bodyweight.

Trixylyl phosphate [Category 1B; H360f]	**TPP**
A combined study of repeated dose and reproductive toxicity showed effects on the testes (a significant and dose-related reduction in testicular weight).	Effects on the testes are associated with marked general toxicity (bodyweight effects); other effects on the male reproductive tract are associated with general toxicity.
Effects were associated with a clear reduction in fertility (reduced numbers of implantations) but were only associated with relatively mild general toxicity (changes in clinical chemistry parameters and reversible effects on organ weights).	A reduction in fertility seen at the highest dose level in the one-generation bolus dosing study was associated with relatively marked general toxicity (bodyweight effects) whereas this effect was not observed at the highest dose level in the two-generation dietary study,

Previous RAC opinions showed that the following were key considerations for deciding that Category 1b classification was more appropriate:

- Potent effects on sexual function or fertility in the absence of general toxicity

- Human epidemiological data demonstrating adverse effects on reproduction parameters

Category 2 Decisions

Indium phosphide [Category 2; H361f]	**TPP**
Effects in the hamster were characterised by reduced testes weight, reduced epididymal weight, testicular histopathology (degeneration of the germinal epithelium) and a reduced sperm count. Effects were also seen in the rat and mouse but only at dose levels causing marked toxicity.	Effects on the male reproductive tract are shown in one species (the rat) but not in the other species investigated (the dog). More minor effects on the male reproductive tract (accessory organs) were observed at lower dose levels and were associated with general toxicity (bodyweight effects).
Effects on the testes were accompanied by general toxicity, however there is no indication that effects were secondary and were therefore considered to be relevant for classification.	Effects on the testes were only apparent at dose levels causing evidence of systemic toxicity. Less marked effects on accessory sex organs were also associated with general toxicity.
Effects on fertility have not been investigated.	A reduction in fertility seen at the highest dose level in the one-generation study (oral bolus gavage) but similar effects were not seen at the highest dose level in the two-generation study (dietary route).

Lucirin [Category 2; H361f]	**TPP**
Repeated dose toxicity studies in the rat demonstrate treatment- and dose-related effects on the testes (reduced weight, atrophy) and reduced spermatogenesis (vacuolar degeneration of spermatogonia, oligospermia, azoospermia). Reduced spermiogenesis was observed in the absence of general toxicity in a 90-day study.	Effects on the male reproductive tract were associated with general systemic toxicity. Inconsistent effects were observed on sperm count, at dose levels causing relatively marked effects on bodyweight.
Effects on fertility have not been investigated	A reduction in fertility seen at the highest dose level in the one-generation oral bolus gavage study at doses that could be predicted to overload hepatic detoxification mechanisms but similar effects were not seen at the highest dose level in the two-generation dietary study.
Effects on the testes were seen in one 28-day study but not in a second study; it is suggested that effects may have been due to a bolus effect.	The absence of effects on fertility in the two-generation (dietary) study at dose levels sufficient to cause bodyweight effects comparable to those seen at the highest dose level in the one-generation (oral gavage) study indicate a bolus effect in the latter study

Previous RAC opinions showed that the following were key considerations for deciding that Category 2 classification was more appropriate:

- Effects on reproduction function were seen only at doses that caused systemic toxicity
- Inconsistencies in findings between studies that could be attributable to a bolus dosing effect
- Deficiencies in studies that cast doubt on the quality of the findings or relevance to humans

b) Developmental effects (Annex I: 3.7.2.4. *Maternal toxicity*)

Classification for reproductive toxicity (adverse effects on development of the offspring) is based on adverse effects induced during pregnancy or as a result of parental exposure. Effects may be

manifested as death of the developing organism, structural abnormality, altered growth or functional deficiency. The relevant studies to consider are therefore studies investigating reproductive toxicity or developmental toxicity.

Criteria for Category 2: Suspected 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 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.

Key consideration:

Developmental effects observed following administration of TPP were clearly associated with marked maternal toxicity and are considered to represent a secondary, non-specific effect. Classification in Category 2 for developmental toxicity is therefore considered not to be appropriate for TPP.

Criteria for Category 1B: Presumed reproductive toxicant

The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect 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.

Developmental effects observed following administration of TPP were clearly associated with marked maternal toxicity and are considered to represent a secondary, non-specific effect. Classification in Category 1b for developmental toxicity is therefore considered not to be appropriate for TPP.

Criteria for Category 1A: Known human reproductive toxicant

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

There is no information available which supports a known adverse effect of TPP in humans.

Summary: Due to marked maternal toxicity concomitant with observed malformations and variations in the offspring, assignment of TPP to this classification category is therefore not appropriate.

3.7.2.2.2 Substances causing effects on or via lactation

Effects on or via lactation

Effects on or via lactation are allocated to a separate single category. It is recognised that, for many substances, there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the basis of:

(a) human evidence indicating a hazard to babies during the lactation period; and/or

(b) the results of one or two generation studies in animals which 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

(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

Classification is appropriate for substances which are absorbed by women and have been shown to interfere with lactation. This relates to effects in the mother that impact adversely on the breast milk, either in terms of the quantity produced or the quality of the milk produced (i.e. the composition). Any effect on the quantity or quality of the breast milk is likely to be due to systemic effects in the mother. However, overt maternal toxicity may not be seen (e.g. the substance may just affect the transfer of a nutrient into the milk with no consequence for the mother). The type and magnitude of the maternal effects and their potential influence on lactation/milk production need to be considered on a case-by-case basis to determine whether classification for effects on or via lactation is necessary. If a substance causes marked overt systemic toxicity in the mother at the same dose level then it is possible that this may indirectly impair milk production or impair maternal care as a nonspecific secondary effect. The type and magnitude of the maternal effects and their potential influence on lactation/milk production needs to be considered on a case-by-case basis using expert judgment. If there is robust evidence to indicate that the effects on lactation are not caused directly by the substance then it should not be classified as such. A substance which does not cause overt toxicity in the mother but which interferes with milk production or quality will normally be classified for effects on or via lactation because in this case the effect on lactation is most likely a direct substance-related effect.

Substances are also classified where there is evidence that may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child. This relates to the ability of the substance (including metabolites) to enter the breast milk in amounts sufficient to cause a concern. When the effect on the offspring is caused by the substance (or metabolite) after transport through the milk then the maternal toxicity has no relevance for classification. In general, positive data should usually be available to show that a substance leads to an adverse effect in offspring due to effects on lactation to support classification. However, in exceptional circumstances, if there are substantiated grounds for concern that the substance may have an adverse effect via lactation then it may be classified as such in the absence of direct evidence. This should be based on a quantitative comparison of the estimated transfer via the milk and the threshold for toxicity in the pups. This might apply in cases where the substance has the capacity to bioaccumulate which would lead to a potentially higher burden in the offspring, or where there is evidence that the offspring may be more sensitive to the substance's toxicity than adult. The mere presence of the substance in the milk alone, without a strong justification for a concern to offspring, would normally not support classification for effects on or via lactation.

4.11.6 Conclusions on classification and labelling

Relevant data are extracted from the repeated dose systemic and reproductive toxicity studies performed with TPP. Data are summarised and compared against the criteria for classification for reproductive toxicity under the CLP Regulation, and published opinions of the Committee for Risk Assessment (RAC) concerning substances classified for reproductive effects. Based on a consideration of all of these factors it is concluded that TPP is most appropriately classified under CLP Regulation as:

Reproductive toxicity (adverse effects on sexual function and fertility): Category 2 [H361f], CLP; Category 3 (R62); DSD

Reproductive toxicity (adverse effects on development of the offspring): Not classified

In the absence of any clear indication of any effect of phenol, dodecyl-, branched, no classification is proposed for effects on or via lactation.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

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

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

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

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

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

Comments received during public consultation

Four MSCAs commented that they do not agree with the proposal to classify TPP in

Category 2 for fertility but that the data presented would support Category 1B. One of these MSCAs further said that the justification for the proposal was poorly argued, and one stated that in addition classification for developmental toxicity is justified.

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

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

Five IND stakeholders commented that they supported the proposed classification as Category 2.
One IND stakeholder (Chevron Oronite, SAS, DS of the other TPP CLH dossier) stated that they did not agree with Category 2 for fertility, but instead support Category 1B. They further submitted additional data, from studies not included in the SI group-UK, Ltd dossier, to support their statement.
One MSCA commented that the substance identity as well as the composition should be clarified.

Assessment and comparison with the classification criteria

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

Adverse effects on sexual function and fertility

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

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

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

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

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

Table 9. Effects on Female reproductive parameters in F0 animals, key findings (Edwards *et al*., 2012*)*

Parameter	Dose Level (mg/kg bw/day)

F0 females and offspring	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 10. Effects on Female reproductive parameters – F1 animals, key findings (Edwards *et al*., 2012)

Parameter F1 females and offspring	Dose Level (mg/kg bw/day)			
	0	1.5	15	75
Mean absolute organ weights and microscopic findings (incidences)				
Mean terminal body weight (g)	413	389	383	315** (↓2
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 11. Effects on Male reproductive parameters – F0 animals, key findings (Edwards *et al*., 2012)

F0 Males (F1 offspring)	**Dose Level (mg/kg bw/day)**			
	0	**1.5**	**15**	**75**
Mean organ absolute weights and microscopic findings (incidences)				
Mean terminal body weight (g)	616	623	611	502** (↓18.5
Mean testes weight (g) left	1.79	1.69	1.75	1.62* (↓5%
Mean testes weight (g) right	1.78	1.74	1.70	1.66
Mean epididymis weight (g) left	0.75	0.72	0.76	0.63** (↓16
Mean epididymis weight (g) right	0.79	0.76	0.79	0.68** (↓13.
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** (↓2
Mean cauda epididymis weight (g) right	0.3671	0.3529	0.3686	0.2838** (↓2
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)	0.060	0.057	0.061	0.057

right				
Mean cauda epididymis weight relative to brain weight (g/100g)left	16.892	15.530	17.450	12.818** (↓24%)
Mean cauda epididymis weight relative to brain weight (g/100g) Right	16.885	16.483	17.137	13.235** (↓22%)
Mean prostate weight (g)	1.13	1.09	1.09	0.88** (↓22%)
Mean prostate weight relative to brain weight (g/100g)	51.959	50.983	50.633	41.039** (↓21%)
Mean seminal vesicle weight (g)	2.34	2.22	2.31	1.74** (↓26%)
Mean seminal vesicle weight relative to body weight (g/100g)	0.404	0.359	0.379	0.346 (↓14%)

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

Table 12. Effects on Male Reproductive Parameters – F1 animals, key findings (Edwards *et al*., 2012)

Parameter F1 Males	**Dose Level (mg/kg bw/day)**			
	0	**1.5**	**15**	**75**
Mean organ absolute weights and microscopic findings (incidence)				
Mean terminal body weight (g)	791	814	754	566** (↓28.4%
Mean body weight (g) - initiation of mating	543	545	536	449** (↓17.3%
Mean testes weight (g) left	1.87	1.94	1.94	1.74
Mean testes weight (g) right	1.93	1.96	1.88	1.72** (↓11%
Mean epididymis weight (g) left	0.67	0.73	0.75* (↑12%)	0.65
Mean epididymis weight (g) right	0.76	0.80	0.77	0.68** (↓10.5%
Epididymis sperm concentration (x106/g) left	310.1	339.4	350.2	320.5
Mean cauda epididymis weight (g) left	0.3028	0.3362	0.3391* (↑12%)	0.2740
Mean cauda epididymis weight (g) right	0.3349	0.3588	0.3372	0.2879* (↓14%
Mean cauda epididymis weight relative to body weight (g/100g) left	0.039	0.042	0.046**	0.049* (↑25%
Mean cauda epididymis weight relative to body weight (g/100g) right	0.043	0.045	0.045	0.052* (↑21%
Mean cauda epididymis weight relative to brain weight (g/100g) left	13.751	15.663* (↑14%)	15.825** (↑15%)	13.116
Mean cauda epididymis weight relative to brain weight (g/100g) right	15.253	16.714	15.720	13.815
Mean prostate weight (g)	1.06	1.07	1.06	0.92* (↓1
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** (↓
Mean seminal weight relative to body weight (g/100g) right	0.28	0.284	0.296	0.32** (↑14%

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

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

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

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

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

<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 13. One-generation study - findings in parental animals

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

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

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

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

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

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

It is concluded that in this one-generation study the mating index was unaffected at all doses, but the fertility index was reduced to 13.3% (93.3% in control group) at 125

mg/kg bw/day. Moderate maternal toxicity was noted at 125 mg/kg bw, as can be inferred based on 9.7% reduction of body weight of parental females at the initiation of mating. Markedly reduced fertility at 125 mg/kg indicates that TPP at a dose moderately toxic to rats can affect fertility.

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

<u>Summary of effects on male fertility:</u>

In the opinion of the DS, the profile of male reproductive changes induced by TPP is consistent with the profile of changes reported for reproductive effects due to food restriction in male rats. Both in the feed restriction studies and in the TPP reproduction and repeated-exposure studies, there was a decrease in accessory reproductive organ weights which was relatively proportionate to the decrease in body weight. For this reason, RAC did not base the classification for fertility on the effects seen in males.

In the two-generation study (Edwards *et al*., 2012), test substance-related organ weight changes at 75 mg/kg bw/day consisted of lower weights of the left and right epididymides (14-16% of control values) and cauda epididymides (by 23-25%), prostate

(21%), and seminal vesicles (26 -17 %)/coagulating glands in F0 and F1 males, and lower left and right testes weights in F1 males. Mean epididymal sperm concentration was also lower in the 75 mg/kg bw/day dose group. These changes occurred together with reduced body weight. The reduction in terminal body weight of male rats was 18.5% in the F0 and 28.4% in the F1, relative to the concurrent control, which is of similar magnitude to the reductions observed in the male accessory sex organ weights relative to the control values (10.5% to 25%, respectively). Consequently there were few statistically significant differences when accessory reproductive organ weights were evaluated relative to control values.

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

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

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

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

In the 28-day repeated dose toxicity study in rats (Harriman, 2004), mean testes weights were statistically significantly reduced by 42% in males at 300 mg/kg bw/day accompanied by germ cell depletion and interstitial cell atrophy. Mean testes weights were reduced by 15% in males at 180 mg/kg bw/day, and although the reduction was not statistically significant, it was accompanied by interstitial cell atrophy (0/5, 0/5, 0/5, 5/5, and 4/5) and depletion of mature germ cells (0/5, 0/5, 0/5, 1/5, 4/5). There was also a low (1/5) incidence of animals with microscopic degeneration of the seminiferous

tubules in the testes at all dose levels, although this effect showed no dose-response over the 5 to 300 mg/kg bw/day dose range.

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

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

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

Several publications which have examined the relationship between body weight changes and male reproductive organ weight changes in the rat (Scharer, 1977; Chapin *et al*., 1993; Levin *et al*., 1993; Keenan *et al*., 1994; Seki *et al*., 1997; Odum *et al*., 2001; Marty *et al*., 2003; Carney *et al*., 2004; Terry *et al*., 2005; Laws *et al*., 2007) have been summarized in OECD draft guidance document 151 (http://www.oecd.org/env/ehs/testing/GD%20151_Oct%202012_clean2.pdf).

These studies showed that reductions in the weights of testes and epididymides were usually smaller than reductions in body weight. A 15% body weight reduction was correlated with a testes and epididymides weights reduction of 2-12%; a 40% body weight reduction resulted in testes and epididymides weights being reduced by 24%. Prostate and seminal vesicle weight varied more with body weight. At 10% body weight reduction, prostate and seminal vesicle weights were reduced by 0-20% and at 40% body weight reduction, prostate and seminal vesicle weights were reduced by 20-45%.

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

Mechanistic Studies Related to Reproductive Toxicity

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

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

Dose-dependent increases in wet (181% - 739%) and blotted (183% - 275%) mean uterine weights at all exposure levels were reported when compared to the vehicle control group. The positive control substance (17α-ethynylestradiol) also elicited the expected increase in uterine weights (wet and blotted), but the percentage of the

increase was not provided for that group.

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

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

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

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

Summary of effects in the uterotrophic bioassays:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The study authors concluded that TPP "exhibited estrogenic effects" in the 60, 250, and 1000 mg/kg bw/d groups based on the early attainment of vaginal patency, early

occurrence of the first oestrus and decreased mean ovary weights.

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

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

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

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

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

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

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

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

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

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

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

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

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

In the CLH report submitted by Chevron Oronite, SAS, several studies were presented in which the test materials used were TPP-derived chemical mixtures containing TPP as an impurity. More details on these studies can be found in the Background document and opinion for the TPP CLH dossier submitted by Chevron Oronite, SAS. Since the detailed composition of the mixtures used in these studies and the purpose of investigating reproductive toxicity of these mixtures are unknown, RAC is of the opinion that the results of these studies have very limited relevance for classification of TPP for reproductive toxicity, therefore these studies will not be considered for justification of a harmonized classification for TPP or for justification of SCL for this substance.

Developmental toxicity

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

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

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

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

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

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

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

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

Table 14. One-generation study: findings in offspring *(taken from CLH report of SI group-UK, Ltd on TPP)*

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

	Day 7	M	15.9	16.1	14.7	14.1
		F	14.6	15.3	13.5	16.9
	Day 14	M	33.0	33.5	29.9**	22.5
		F	31.2	32.3	28.0**	29.3
	Day 21	M	52.5	53.0	47.5**	34.5
		F	49.4	50.8	44.8**	46.1
Balano-preputial separation (day)		M	43.2	42.9	44.6*	47.5
Balano-preputial separation (g)			230.1	226.0	229.1	205.7
Vaginal patency (day)		F	33.0	32.8	33.5	32.5
Vaginal patency (g)			115.1	116.0	110.2	110.6

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

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

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

Summary of the classification justification

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

secondary non-specific consequences of other toxic effects.

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

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

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

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

Based on the *in vitro* rat prostate androgen receptor competitive binding assay (Thomas *et al*., 2012a) TPP is considered an AR binder. The binding affinity of TPP was similar to the weak positive control, dexamethasone.

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

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

4.12 Other effects

No additional information is available.

4.12.1 Non-human information

No additional information is available.

4.12.1.1 Neurotoxicity

The chemical structure of phenol, dodecyl-, branched has no structural relationships with any known neurotoxicants. A review of the available toxicity data shows no evidence of clinical signs indicative of neurotoxicity or neuropathological changes in the acute, sub-acute or sub-chronic toxicity studies. No classification for phenol, dodecyl-, branched for specific target organ toxicity (STOT-SE or STOT-RE) is proposed, in the absence of any specific (structural or experimental) indication of neurotoxicity.

4.12.1.2 Immunotoxicity

A review of the available toxicity data shows no evidence of immunotoxicity (organ weight changes or histopathology) in the sub-acute or sub-chronic toxicity studies. No classification for phenol, dodecyl-, branched for specific target organ toxicity (STOT-SE or STOT-RE) is proposed, in the absence of any specific (structural or experimental) indication of neurotoxicity.

No additional information available.

4.12.1.3 Specific investigations: other studies

No additional information is available.

4.12.1.4 Human information

No additional information is available.

4.12.2 Summary and discussion

Based on the available toxicological information, there are no other effects of phenol, dodecyl-, branched which would trigger classification according to the criteria of Regulation (EC) No. 127/2008.

4.12.3 Comparison with criteria

Not relevant.

4.12.4 Conclusions on classification and labelling

Based on the available toxicological information, there are no other effects of phenol, dodecyl-, branched which would trigger classification according to the criteria of Regulation (EC) No. 127/2008.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

No study is available on the basis that it is technically not feasible. In accordance with Annex XI Section 2 of the REACH Regulation EC 1907/2006, testing for a specific endpoint may be omitted, if it is technically not possible to conduct the study as a consequence of the properties of the substance. The REACH Regulation notes that degradation testing is not required for substances that are highly volatile from water and thus cannot be kept in solution under the experimental conditions of this test. It is also noted that studies may be difficult to conduct with substances of minimal solubility in water.

Phenol, dodecyl-, branched is of very low water solubility (1.54 mg/L) which is considered to affect the technical feasibility of a study. Using a theoretical assessment based on the composition and the structural formula, it can be concluded that destructive hydrolysis of the substance will not be evident at any pH (i.e. the substance will not be degraded by hydrolytic means in either strong aqueous acid or strong base). The substance also consists of a structural moiety (phenolic hydroxyl group), which will react reversibly (be neutralized) when any water soluble fraction is contacted with especially strong bases (pH>11). This reaction is not destructive and is reversible upon acidification of the aqueous media employed. Apart from this consideration, there are no functional groups or other structural alerts present in the active organic ingredient that would indicate that this substance would itself hydrolyse in an irreversible manner, and thus it should not be classified according to the criteria for hydrolysis as a function of pH. Furthermore, this is confirmed by long term handling experience.

Table 49: Summary of relevant information on degradation

Method	Results	Remarks	Reference
OECD Method 111 (hydrolysis of a function of pH)	Not applicable	Scientifically and technically the study is unfeasible and unjustified	NA

5.1.1 Stability

No data are available. The substance is predicted to be chemically stable.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

Table 50: Overview of screening tests for biodegradation in water

Method	Results	Remarks	Reference
Test type: inherent biodegradability sewage, predominantly domestic (adaptation not specified) OECD Guideline 302 D (Inherent Biodegradability-Concawe Test)	not inherently biodegradable % Degradation of test substance: 10% after 56 d (CO_2 evolution)	key study, experimental result Test material (EC name): Phenol, dodecyl-, branched, CAS 74499-35-7	Mead & McKenzie (2005)
Test type: ready biodegradability sewage, predominantly domestic, non-adapted OECD Guideline 301 B (Ready Biodegradability: CO2 Evolution Test)	under test conditions no biodegradation observed % Degradation of test substance: 25% after 28 d (CO_2 evolution) (starting conc. 10 mg/L) 6% after 28 d (CO_2 evolution) (starting conc. 20 mg/L)	key study, experimental result Test material (EC name): Phenol, dodecyl-, branched. CAS 121158-58-8	Schörberl P (1992a)

Biodegradation study:

A study was performed to assess the inherent biodegradability of the test material in an aerobic aqueous medium. The method followed the recommendations of CONCAWE (October 1999) 'A Test Method to Assess the Inherent Biodegradability of Oil Products' and the draft OECD Test Guideline (October 2001) No 302D 'Inherent Biodegradability: CONCAWE Test'.

The substance, at a concentration of 20 mg C/L, was exposed to activated sewage sludge micro-organisms with culture medium in sealed culture vessels in the dark at 20 ±1°C for 56 days. Data supplied by the Sponsor indicated that the bulk solubility of the test material was 1.1 mg/L, therefore following the recommendations of the Test Guidelines for dealing with insoluble test materials, the test material was dissolved in a volatile organic solvent and an aliquot of the solvent stock solution applied to a glass fibre filter paper. After evaporation of the solvent, the filter paper containing the test material was added to the test medium. The degradation of the test material was assessed by the determination of carbon dioxide produced on Days 0, 1, 2, 3, 6, 8, 10, 14, 21, 28, 35, 42, 49 and 56; and by compound specific analyses on Days 0, 1, 2, 3, 6, 8, 10, 14, 21, 42 and 56. Control solutions with inoculum and the standard material, n-hexadecane, together with a toxicity control were used for validation purposes.

The test material attained 10% biodegradation after 56 days based on carbon dioxide production and therefore cannot be considered to be inherently biodegradable. The results of the compound specific analyses indicated that no significant chemical or biological degradation of the test material occurred.

5.1.2.3 Simulation tests

No data are available.

5.1.3 Summary and discussion of degradation

The substance is neither readily nor inherently biodegradable according to the two key studies.

Ready biodegradability

Phenol, dodecyl-, branched showed limited biodegradation (25% degradation at 10 mg/L and 6% at 20 mg/L) over 28 days in a ready (OECD 301B) biodegradation study (Schörberl, 1992a). The study was conducted using unadapted inoculum from a municipal sewage plant. The positive control, sodium benzoate, attained 95% degradation after 28 days indicating viability of the inoculum. The results of this study indicate that the substance is not readily biodegradable.

Inherent biodegradability:

A study was performed to assess the inherent biodegradability of the substance in an aerobic aqueous medium. The method followed the recommendations of CONCAWE (October 1999) 'A Test Method to Assess the Inherent Biodegradability of Oil Products' and the draft OECD Guideline (October 2001) No 302D 'Inherent Biodegradability: CONCAWE Test'. The test material attained 10% biodegradation after 56 days based on carbon dioxide production and therefore cannot be considered to be inherently biodegradable. The results of the compound specific analyses indicated that no significant chemical or biological degradation of the test material occurred. The results of this study therefore indicate that phenol, dodecyl-, branched is not inherently biodegradable.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Table 51: Overview of studies on adsorption/desorption

Method	Results	Remarks	Reference
Study type: adsorption (a single test at approximately neutral pH on the unionised form was performed.) HPLC estimation method OECD Guideline 121 (Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)) EU Method C.19 (Estimation of the Adsorption Coefficient (KOC) on Soil and Sewage Sludge Using High Performance Liquid Chromatography (HPLC))	Adsorption coefficient: log Koc: 0.000104-0.000471 at 30 °C	key study, experimental result Test material (Common name): Tetrapropenyl Phenol	Woolley & O'Connor (2010)

Adsorption coefficients

The Adsorption Coefficient was determined to be 10.4-4.71 $x10^4$ ($log_{10}K_{oc}$1.02-4.67), using the HPLC screening method, designed to be compatible with Method C19 Adsorption Coefficient of Council Regulation (EC) No 440/2008 of 30 May 2008 and Method 121 of the OECD Guidelines for Testing of Chemicals, 22 January 2001. However, 93.9% of the test item (the dominant

components) had a reduced range of 2.49-4.71 $x10^4$ ($log_{10}K_{oc}$4.40-4.67) using the HPLC screening method, designed to be compatible with Method C19 Adsorption Coefficient of Council Regulation (EC) No 440/2008 of 30 May 2008 and Method 121 of the OECD Guidelines for Testing of Chemicals, 22 January 2001.

5.2.2 Volatilisation

No data are available. The low vapour pressure of 1.1 $x10^{-2}$ Pascals at 25°C indicates little potential for volatilisation of the substance.

5.2.3 Distribution modelling

No data are available.

5.3 Aquatic Bioaccumulation

Table 52: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Oncorhynchus mykiss aqueous (freshwater), flow-through Total uptake duration: 27 d Total depuration duration: 15 d Details of method: No data OECD Guideline 305 (Bioconcentration: Flow-through Fish Test) EPA OPPTS 850.1730 (Fish BioconcentrationTest)	BCF: 289 dimensionless (edible fraction) (Time of plateau: 3 d)(steady state) (Based on total radioactivity) BCF: 1601 dimensionless (non-edible fraction) (Time of plateau: 3 d)(steady state) (Based on total radioactivity) BCF: 823 dimensionless (whole body w.w.) (Time of plateau: 3 d)(steady state) (Based on total radioactivity) BCF: 289 dimensionless (edible fraction) (Time of plateau: 3 d)(steady state) (Based on total radioactivity) BCF: 1428 dimensionless (non-edible fraction) (Time of plateau: 3 d)(steady state) (Based on total radioactivity) BCF: 749 dimensionless (whole body w.w.) (Time of plateau: 3 d)(steady state) (Based on total radioactivity) Elimination: yes; During depuration, TPP was eliminated quickly with mean tissue concentrations 10% or less than the mean measured steady-state test concentrations by Day 11.: 11 d Lipid content: 4-5.45 % (end of exposure) (Edible Tissue Samples) 7.87-14.2 % (end of exposure) (Non-edible Tissue Samples)	Key study, experimental result Test material: Phenol, dodecyl-, branched, CAS 74499-35-5	A. Blankinship, T.Z. Kendall & H.O. Krueger (2006)

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Steady-state TPP concentrations were achieved in the tissues of rainbow trout (*Oncorhynchus mykiss*) after 3 days for both the 1.1 and 11 µg/L treatment groups.

Steady-state BCF values based on total radioactivity TPP concentrations were 289, 1601 and 823 in edible, non-edible and whole fish tissue, respectively, for the 1.1 µg/L treatment group, and 289, 1428 and 749 in edible, non-edible and whole fish tissue, respectively, for the 11 µg/L treatment group. Steady-state BCF values based on TPP chain lengths for the 1.1 µg/L treatment group were 209 and 544 for the C5-C10 edible and non-edible fraction, 362 and 786 for the C11-C12 edible and non-edible fraction, and 426 and 987 for the C13 and greater edible and non-edible fraction, respectively. Steady-state BCF values were 217 and 603 for the C5-C10 edible and non-edible fraction, 388 and 845 for the C11-C12 edible and non-edible fraction, and 423 and 936 for the C13 and greater edible and non-edible fraction, respectively for the 11 µg/L treatment group. During depuration, TPP was eliminated quickly with mean tissue concentrations 10% or less than the mean measured steady-state test concentrations by Day 11.

5.3.1.2 Measured bioaccumulation data

No measured data are available.

5.3.2 Summary and discussion of aquatic bioaccumulation

Steady-state TPP concentrations were achieved in the tissues of rainbow trout (*Oncorhynchus mykiss*) after 3 days. During depuration, TPP was eliminated quickly with mean tissue concentrations 10% or less than the mean measured steady-state test concentrations by Day 11.

5.4 Aquatic toxicity

Table 53: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
Freshwater, semi-static Acute Toxicity Test *Pimephales promelas* OECD Guideline 203 (Fish, Acute Toxicity Test)	EL_{50} (96 h): 40 mg/L nominal phenol, dodecyl-, branched NOELR (96 h): 25 mg/L nominal phenol, dodecyl-, branched	Key study, experimental result Test material: Phenol, dodecyl-, branched, CAS 74499-35-5. Purity not reported	Boeri, Ward & Kowalski (1994)
Freshwater, static, Acute Toxicity Test. *Pimephales promelas* equivalent or similar to OECD Guideline 203 (Fish, Acute Toxicity Test)	LC_{50} (96 h): 3.2 mg/L nominal phenol, dodecyl-, branched	Supporting study, experimental result Test material: Phenol, dodecyl-, branched, CAS 74499-35-5. Purity not reported	Ward, Kowalski & Boeri (1994)
Feshwater, static. Acute Toxicity Test, *Pimephales promelas* EPA-660/3-75-009, April, 1975	LC_{50} (96 h): 24 mg/L nominal phenol, dodecyl-, branched	Supporting study, experimental result Test material: Phenol, dodecyl-, branched, CAS 74499-35-5. >99% purity	Griffen & Thompson (1981)
Leuciscus idus melanotus Freshwater, semi-static. EU Method C.1 (Acute Toxicity for Fish) (Cited as Directive 84/449/EEC, C.1 ('Acute toxicity for fish'))	Analytical monitoring was performed in this study, but the concentrations could not be measured because they were below the detection limit of the analytical method employed (0.5 mg/L).	Supporting study experimental result Test material: phenol, dodecyl -, branched (CAS 121158-58-5 99.9% purity	Scholz, N (1993) OECD SIDS (2006)

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

In the key study for this endpoint, the acute toxicity of the water accommodated fraction (WAF) of 6.3, 13, 25, 50, and 100 mg/L mixtures of the substance and water to the fathead minnow, *Pimephale promelas*, was investigated. Analytical confirmation of exposure concentrations is not provided, but this study was conducted most recently and had fewer deviations than the supporting studies and was therefore chosen as the most reliable study. Effect levels were reported in terms of concentration, however as WAFs (water accommodation fraction) were used, the results should be reported in terms of loading levels (i.e. NOELR and EL_{50}).

The test, which was designed to determine the toxicity of the WAFS of the test substance, was performed from February 8 to 12, 1994. (OECD Guideline 203 (Fish, Acute Toxicity Test)). The test was performed at 22 ±1°C under static, renewal conditions with a control and the WAFs of five concentrations of the test substance. The dilution water was carbon-filtered, dechlorinated tap water adjusted to a hardness of 160-180 mg/L. Juvenile fathead minnows were procured from a commercial supplier and acclimated to test conditions for at least 14 days prior to use in the test. At the conclusion of the test control fish had an average wet weight of 0.21 g and an average total length of 29 mm. The five WAFS were prepared by formulating five concentrations of the

substance and dilution water (6.3, 13, 25, 50, and 100 mg/L) in glass mixing vessels equipped with a magnetic stirrer, stirring the mixtures for approximately 24 hours, settling the mixtures for approximately 1 hour, and siphoning the water phase containing the WAF. No insoluble material was noted in any of the test vessels during the study.

The 96 hour median lethal concentration (LC_{50}) of the WAFs of the test material to fathead minnows was 40 mg/L (expressed as the nominal amount of test substance used to prepare the WAF) with a 95% confidence interval of 25-50 mg/L. The estimated no observed effect concentration (NOEC) is 25 mg/L.

Supporting studies are also available for this endpoint:

Ward, Kowalski & Boeri, (1994): the substance is highly insoluble in water and it was considered that the method used to disperse the test material was inappropriate and therefore this study is considered unreliable and will not be used for classification purposes. Test vessels containing 10, 35, and 100 mg/L of the test material had insoluble material on the surface and were cloudy (cloudiness increased with concentration). Exposure of fathead minnows to the test material resulted in a 96-hour median lethal concentration (LC_{50}) of 3.2 mg/L (95% confidence interval 1.0-10 mg/L). The no observed effect concentration (NOEC) was estimated to be 1.0 mg/L.

Griffen & Thompson, (1981): the substance is highly insoluble in water and it was considered that the method used to disperse the test material was inappropriate and therefore this study is considered unreliable and will not be used for classification purposes. An oily film was observed on the surface of each test chamber after addition of the test material. The observed 96-hour LC_{50} value and 95% confidence limits was 24 mg/L (23-30 mg/L). The results indicated a 96-hour NOEC of 5.6 mg/L.

Scholz, (1993): this study lacked detail in the report on the preparation of the test solution. The substance was not toxic to fish when prepared as a saturated solution with an analytical concentration of <0.5 mg/L.

On the basis of the results of the key study, the LC_{50} for freshwater fish is 40 mg/L

5.4.1.2 Long-term toxicity to fish

No data are available.

5.4.2 Aquatic invertebrates

Table 54: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
Short-term toxicity to aquatic invertebrates			
Freshwater, static, *Daphnia magna*. OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) EU Method C.2 (Acute Toxicity for Daphnia)	EC50 (48 h): 0.037 mg/L nominal tetrapropenyl Phenol, based on mobility	Key study, experimental result. Test material: tetrapropenyl phenol	Sewell & McKenzie (2005a)
Freshwater, static, *Daphnia magna*. OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	EL_{50} (48 h): 3.4 mg/L nominal test material Phenol, dodecyl-, branched, based on mobility NOELR (48 h): 1 mg/L test mat.	Supporting study, experimental result Test material: tetrapropenyl phenol	Ward & Magazu (1994)

Method	Results	Remarks	Reference
EPA OTS 797.1300 (Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids)	(nominal) based on: mobility	CAS 74499-35-7	
Daphnia magna freshwater static Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (US EPA, 1975)	EC_{50} (48 h): 0.072 mg/L nominal test material Phenol, dodecyl-, branched, based on mobility	Supporting study, experimental result Test material: Phenol, dodecyl (CAS 27193-86-8)	Calvert & Adams (1981)
Daphnia magna freshwater static EU Method C.2 (Acute Toxicity for Daphnia)	EC_{50} (48 h): 0.093 mg/L nominal test material Phenol, dodecyl-, branched, based on mobility	Supporting study, experimental result Test material: Phenol, dodecyl (CAS 27193-86-8)	Scholz, N (1992a) OECD SIDS(2006)
Mysidopsis bahia (new name: Americamysis bahia) saltwater static EPA OTS 797.1930 (Mysid Acute Toxicity Test)	EL_{50} (96 h): 0.58 mg/L nominal test material Phenol, dodecyl-, branched, based on mobility NOELR (96 h): 0.46 mg/L nominal test material Phenol, dodecyl-, branched, based on mobility EL50 (96 h): 57 mg/L nominal test material Phenol, dodecyl-, branched, based on lethargy	Supporting study, experimental result Test material: phenol, dodecyl -, branched (CAS 121158-58-5 100% purity	Simon, K (1998)
Long-term toxicity to aquatic invertebrates			
Freshwater, semi-static, *Daphnia magna* OECD Guideline 211 (*Daphnia magna* Reproduction Test) EU Method C.20 (*Daphnia magna* Reproduction Test)	NOEC (21 d): 0.0037 mg/L nominal test material Phenol, dodecyl-, branched, based on immobilisation LOEC (21 d): 0.012 mg/L nominal test material Phenol, dodecyl-, branched, based on immobilisation	Key study, experimental result Test material: Phenol, (tetrapropenyl) derivatives (CAS No. 74499-35-7). Purity not provided	Sewell & McKenzie (2005b)
Toxicity to aquatic plants			
Freshwater, static, *Scenedesmus subspicatus (new name: Desmodesmus subspicatus)* (algae) OECD Guideline 201 (Alga, Growth Inhibition Test)	EC_{50} (72 h): 0.15 mg/L nominal test material Phenol, dodecyl-, branched, based on: biomass EC_{50} (72 h): 0.36 mg/L nominal test material Phenol, dodecyl-, branched, based on: growth rate NOEC (72 h): 0.07 mg/L nominal test material Phenol, dodecyl-, branched	Key study, experimental result Test material: Phenol, (tetrapropenyl) derivatives (CAS No. 74499-35-7). Purity not provided	Vryenhoef & McKenzie (2005)
Freshwater, static, *Selenastrum capricornutum (new name: Pseudokirchnerella subcapitata)* (algae) OECD Guideline 201 (Alga, Growth Inhibition Test)	EL_{50} (96 h): 97 mg/L nominal test material Phenol, dodecyl-, branched, based on: cell number EL_{50} (96 h): 240 mg/L nominal test material Phenol, dodecyl-, branched, based on: growth rate	Supporting study, experimental result Test material: Phenol, (tetrapropenyl) derivatives (CAS No. 74499-35-7). Purity	Ward, Boeri & Magazu (1994)

Method	Results	Remarks	Reference
EPA OTS 797.1050 (Algal Toxicity, Tiers I and II)	NOELR (96 h): 62 mg/L nominal test material Phenol, dodecyl-, branched, based on: cell density & growth rate	not provided	
Scenedesmus subspicatus (new name: Desmodesmus subspicatus) (algae) Freshwater, static Algae Growth Inhibition Test, Following EEC G88/302	EL_{50} (72 h): >0.765 mg/L nominal test material Phenol, dodecyl-, branched, based on: growth rate NOELR (72 h): 0.44 mg/L nominal test material Phenol, dodecyl-, branched	Supporting study, experimental result Test material: phenol, dodecyl -, branched (CAS 121158-58-5 100% purity	Scholz, N (1992b) OECD SIDS(2006)

5.4.2.1 Short-term toxicity to aquatic invertebrates

The key study for this endpoint (Sewell & McKenzie, 2005; SPL project number: 1666/027) for acute toxicity to *Daphnia magna*, was conducted according to OECD Guideline 202 (*Daphnia* sp. Acute Immobilisation Test), referenced as Method C.2 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC). Following a preliminary range-finding test, twenty daphnids (2 replicates of 10 animals) were exposed to an aqueous solution of the substance at concentrations of 0.011, 0.020, 0.035, 0.062, 0.11, 0.20, 0.35, 0.62 and 1.1 mg/L for 48 hours at a temperature of approximately 21°C, under static conditions. The numbers of immobilised *Daphnia* were recorded after 24 and 48 hours. The 48-Hour EC_{50} for the test material to Daphnia magna based on nominal test concentrations was found to be 0.037 mg/L with 95% confidence limits of 0.031-0.044 mg/L. The No Observed Effect Concentration (NOEC) was 0.011 mg/L.

The following supporting studies are also available for this endpoint:

Ward & Magazu, (1994): the value of this study is restricted as a consequence of the lack of analytical confirmation of test concentrations. The 48-hour median effective concentration (EC_{50}) of the test material to daphnids (expressed as the nominal amount of test substance use to prepare the WAF) in this study was reported to be 3.4 mg/L (95% confidence intervals 2.8-4.7 mg/L) and the 48-hour NOEC was 1.0 mg/L. Effect levels in this study were reported in terms of concentration, however as WAFs (water accommodation fraction) were used, the results should be reported in terms of loading levels (e. g. NOELR and EL_{50}).

Calvert & Adams, (1981): the value of this study is restricted as a consequence of the lack of analytical confirmation of test concentrations. The 24-hour EC_{50} in this study was found to be 0.074 mg/L. The 48-hour EC_{50} was 0.072 mg/L and the 48-hour NOEC was 0.031 mg/L.

Scholz (1992): information for this study was obtained from the 2006 SIDS dossier; the original study report is unavailable. The reliability of this study is further restricted by the lack of analytical confirmation of exposure concentrations. In addition, acetone was used as a co-solvent at a level of 0.45 ml/L, which exceeds current guidance recommending a limit of 0.1 ml/L. It is possible that the use of acetone at this concentration may have affected the distribution of the different homologues in the test medium. The 24 and 48 hour EC_{50} values were reported to be 0.106 and 0.092 mg/L, respectively.

Simon (1998): the reliability of this study is restricted due to the lack of analytical confirmation of test concentrations and the use of a non-standard species of marine invertebrate. Under the conditions of this study the 96-hour LC_{50} was 0.58 mg/L WAF. The associated NOEC was

0.46 mg/L WAF. Effect levels were reported in terms of concentration, however as WAFs (water accommodation fraction) were used, the results should be reported in terms of loading levels.

The critical value is therefore the EC_{50} for freshwater invertebrates (Daphnia magna) of 0.037 mg/L, reported in the study of Sewell & McKenzie (2005).

5.4.2.2 Long-term toxicity to aquatic invertebrates

The key study (Sewell & McKenzie, 2005) for effects on *Daphnia* reproduction was conducted according to the OECD Guideline 211 (*Daphnia Magna* Reproduction Test) and EU Method C.20. The 14-day and 21-day EC_{50} (immobilisation) values, based on nominal test concentrations, for the parental *Daphnia* generation (P1) were calculated to be 0.0082 (0.0059-0.012) mg/L and 0.0079 (0.0055-0.011) mg/L respectively. The 21-day EC_{50} (reproduction) value, based on nominal test concentrations, was calculated to be 0.0086 (0.0061-0.012) mg/L. The Lowest Observed Effect Concentration (LOEC) was considered to be 0.012 mg/L on the basis of significantly fewer live young per adult ($p<0.05$) produced at this concentration when compared to the solvent control; significant mortality (immobilisation) was also observed in the parental generation (P1) at this test concentration. The NOEC was considered to be 0.0037 mg/L on the basis that at this test concentration there were no significant mortalities (immobilisation) observed in the parental generation (P1) and that there were no significant difference ($P>= 0.05$) between the solvent control and the 0.0037 mg/L test group in terms of numbers of live young produced per adult by Day 21.

The long-term NOEC for freshwater invertebrates is therefore 0.0037 mg/L.

5.4.3 Algae and aquatic plants

The key study (Vryenhoef & McKenzie, 2005) for the algal inhibition to *Scenedesmus subspicatus*, the study was conducted according to the OECD Guideline 201 (Alga, Growth Inhibition Test). Following a preliminary range-finding test, *Scenedesmus subspicatus* was exposed to an aqueous solution of the substance at concentrations of 0.070, 0.14, 0.28, 0.55 and 1.1 mg/L (three replicate flasks per concentration) for 72 hours, under constant illumination and shaking, at a temperature of 24 ±1°C. Samples of the algal populations were removed daily and cell concentrations determined for each control and treatment group, using a Coulter Multisizer Particle Counter. Exposure of *Scenedesmus subspicatus* to the test material gave an EbC_{50} (72 h) value of 0.15 (0.13-0.18) mg/L and an ErC_{50} (0-72 h) value of 0.36 (0.26-0.50) mg/L. The NOEC was 0.070 mg/L.

The following supporting studies are also available for this endpoint:

Ward, Boeri & Magazu, (1994): the reliability of this study is restricted due to the lack of analytical confirmation of test concentrations. Based on the number of cells/ml, the 72-hour EC_{50} is 100 mg/L and the 96 hour EC_{50} is 97 mg/L. Based on the average specific growth rate, the 72-hour EC_{50} is 270 mg/L and the 96-hour EC_{50} is 240 mg/L. Based on the number of cells/mL or the average specific growth rate, the 72- and 96-hour NOEC values are 62 mg/L. Re-growth of inhibited cultures from the 350 mg/L test level revealed that the effect at this concentration of the test material was algistatic. Effect levels in this study were reported in terms of concentration, however as WAFs (water accommodation fraction) were used, the results should be reported in terms of loading levels (e.g. NOELR and EL_{50}).

Scholz, (1992): information for this study was obtained from the 2006 SIDS dossier due to the original report being unavailable. The reliability of the study is further restricted due to the lack of analytical confirmation of exposure concentrations. On the basis of growth rate the 72-hour ErC_{50} was >0.765 mg/L; 10% inhibition of growth rate was attained at >0.765 mg/L. The NOEC was

0.442 mg/L. Effect levels in this study were reported in terms of concentration, however as WAFs (water accommodation fractions) were used, the results should be reported in terms of loading levels (e.g. NOELR and EL_{50}).

Based on the results of the key study, the critical value is the NOEC for freshwater algae of 0.07 mg/L.

5.4.4 Other aquatic organisms (including sediment)

No available information-data generation waived on basis of the chemical safety assessment not indicating a need for further investigation and consequently sediment toxicity testing was not conducted.

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

Aquatic toxicity criteria

Acute aquatic toxicity is normally determined using a fish 96-hour LC_{50}, a crustacean species 48-hour EC_{50} and/or an algal species 72- or 96-hour EC_{50} value. These species cover a range of trophic levels and taxa and are considered as surrogates for all aquatic organisms. Data on other species (e.g. *Lemna* spp.) shall also be considered if the test methodology is suitable. The aquatic plant growth inhibition tests are normally considered as chronic tests but the derived EC_{50} values are treated as acute values for classification purposes.

In toxicity studies for algae and aquatic plants EC_{50} values of concentrations below 1 mg/L were obtained and therefore the algae test was considered to provide the most sensitive acute endpoint:

EC_{50}/LC_{50} for freshwater algae: 0.36 mg/L

EC_{10}/LC_{10} or NOEC for freshwater algae: 0.07 mg/L

Chronic exposure indicated that the Daphnia reproduction study provided the most sensitive chronic endpoint:

Chronic NOEC (21 day) for *Daphnia magna*: 0.0037 mg/L

In addition, phenol, dodecyl-, branched is not readily biodegradable.

Based on these findings, phenol, dodecyl-, branched should be classified with the aquatic environmental hazard acute Category 1, H400 and aquatic environmental hazard chronic Category 1, H410. Based on the acute and chronic toxicity data for *Pseudokirchneriella subcapitata* (ErC_{50} = 0.36 mg/L) and *Daphnia magna* reproduction (0.0037 mg/L) the M-factors are 1 and 10 for acute and chronic classifications respectively, according to the criteria specified in the 2nd ATP.

Concentration Limits according to Annex III Table 1b of Directive 2006/8/EC, based on an LC50 value of 0.36 mg/L.

N; R50-53: $Cn \geq 25$ %
N; R51-53: $2.5\% \leq Cn < 25\%$
R52-53: $0.25\ \% \leq Cn < 2.5\ \%$

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

Using the 'worst case' aquatic toxicity values; *Pseudokirchneriella subcapitata* EC_{50} = 0.36 mg/L (Vryenhoef & McKenzie, 2005) and *Daphnia magna* NOEC = 0.0037 mg/L (Sewell and McKenzie, 2005b) and based on the fact that no biodegradation was observed, the classification for environmental hazards according to Regulation (EC) No. 1272/2008 for phenol, dodecyl-, branched is:

Aquatic Acute Category 1; H400 – Very toxic to aquatic life

Aquatic Chronic Category 1; H410 - Very toxic to aquatic life with long lasting effects

According to DSD criteria, classification with R50/53 'Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment' is proposed.

Based on these findings, phenol, dodecyl-, branched should be classified with the aquatic environmental hazard acute Category 1, H400 and aquatic environmental hazard chronic Category 1, H410. Based on the acute and chronic toxicity data for *Pseudokirchneriella subcapitata* (ErC_{50} = 0.36 mg/L) and *Daphnia magna* reproduction (0.0037 mg/L) the M-factors are 1 and 10 for acute and chronic classifications respectively, according to the criteria specified in the 2nd ATP.

Concentration Limits according to Annex III Table 1b of Directive 2006/8/EC, based on an LC50 value of 0.36 mg/L.

N; R50-53: $Cn \geq 25$ %
N; R51-53: $2.5\% \leq Cn < 25\%$
R52-53: $0.25\ \% \leq Cn < 2.5\ \%$

RAC evaluation of environmental hazards

Summary of Dossier submitter's proposal

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

Comments received during public consultation

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

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

The same MSCA suggested that the reported BCF values should be lipid normalized. In

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

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

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

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

Additional key elements

Two issues were flagged during public comment but did not receive an adequate response from the DS:

1. The available data for branched 4-dodecylphenol have previously been reviewed in detail by the UK Competent Authority (Brooke *et al*., 2007. Environmental risk evaluation report: *para*-C_{12}-alkylphenols (dodecylphenol and tetrapropenylphenol). SCHO0607BMVN-E-P. Environment Agency, Bristol, UK). It was noted that test concentration maintenance in both of the key toxicity tests with *D. magna* was poor (e.g. analysis of centrifuged test samples at 48 h showing measured concentrations 23-49% of nominal in the acute test). The results of the studies were recalculated by the UK Competent Authority based upon geometric/time-weighted mean measured concentrations, giving a 48-h EC_{50} of 0.017 mg/l and 21-day NOEC of 0.002 mg/l, respectively. Similar calculations were performed for the algal test. These values are lower than those based on nominal concentrations, but are of the same order of magnitude. Whilst they do not affect the classification, they are preferred as a more conservative indication of toxicity.

2. No long-term fish toxicity data are available for this substance. Long-term fish growth and reproduction NOECs are typically in the range 0.001 – 0.01 mg/l for branched 4-nonylphenol (a close structural analogue), with markers of oestrogen agonism appearing at similar or lower concentrations (ECHA, 2012. Member State Committee Support Document for Identification of 4-nonylphenol, branched and linear as a Substance of Very High Concern. Adopted on 13 December 2012. European Chemicals Agency). This triggers classification as Aquatic Chronic 1, with an M factor of 10 for a non-rapidly degradable substance. It is possible that branched 4-dodecylphenol can also cause similar effects in fish, but in the absence of data RAC does not think it is appropriate to speculate about the likely magnitude of the long-term fish NOEC/EC_{10} for this substance.

Assessment and comparison with the classification criteria

Degradability: TPP is not expected to hydrolyse under standard conditions at pH 4, 7 or 9. It failed a test for ready biodegradation (achieving at most 25% mineralisation in 28 days). An inherent biodegradability test also indicated a low degree of biodegradation

(10% biodegradation after 56 days). Although the substance may contain some constituents that are more degradable than others, the available information indicates that it is neither rapidly degradable nor readily biodegradable in the environment.

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

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

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

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

Classification according to the CLP Regulation

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

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

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

Classification according to DSD

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

Table 16. Specific concentration limits and classification of the mixture

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

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

6 OTHER INFORMATION

None.

7 REFERENCES

Data point / Reference number	Author(s)	Year	Title	Data protection claimed	Owner
Section 1.3	Woolley & O'Connor	2010	Determination of physico-chemical properties, Harlan Laboratories Ltd, Shardlow, Report No.: 3157/0001. GLP, Unpublished	Y	Calicio Ltd. On behalf of Additives Technical Committee, Task Force 33C
Section 1.3	Oronite Additives	1993	Product specification for tetrapropenyl phenol., Oronite Additives. Report No.: 882 DIESEL. non-GLP, Unpublished	Y	Oronite Additives
Section 1.3	Tremain and Atwal	2010	Determination of vapour pressure GLP, Unpublished	Y	Calicio Ltd On behalf of Additives Technical Committee Task Force 33
Section 4.6.1	Morris	1997	Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Technique). Testing laboratory: Hill Top Research, Ltd., Miarniville, Ohio. Report no.: 96-8243-21. Study number: 96-45. GLP. Unpublished	Y	Chevron Research and Technology Company
Section 1.3	Woolley & O'Connor	2010	Determination of physico-chemical properties., Harlan Laboratories Ltd, Shardlow, Report No.: 3157/0001. GLP, Unpublished	Y	Calicio Ltd. On behalf of Additives Technical Committee, Task Force 33C
Section 1.3	Oronite Additives	1993	Unpublished	Y	Chevron Oronite Company LLC
Section 4.2	Randall D and Robinson E	1978	Acute Toxicologic Evaluation of the test material. Journal of the American College of Toxicology 1(1):72-73. Non-GLP. Published	N	Monsanto Company-Dept. of Med. & Health Sciences
Section 4.2	Mürmann, P	1984a	Acute Oral Toxicity of the test material for rats. Testing laboratory: Hüls Company Ltd. Report no.: Final Report No. 0286. GLP, Unpublished.	Y	American Chemistry Council, Petroleum Additives Panel - HERTG Consortium
Section 4.2	Cavalli, Hallesy and Spence	1968	Toxicity of the test material. Testing laboratory: Industrial Hygiene & Toxicology, Standard Oil Company of California, safety division. Report no.: SOCO 31/I:91	Y	Chevron Chemical Company

Data point / Reference number	Author(s)	Year	Title	Data protection claimed	Owner
			Non-GLP, Unpublished		
Section 4.2	Costello & Gilman	1982	Acute Oral Toxicity-Rats. Testing laboratory: Biosearch, Inc, Philadelphia, Pennsylvania. Report no.: 83-3157A GLP, Unpublished	Y	The Lubrizol Corporation
Section 4.2	Cavalli, Hallesy and Spence	1968	Toxicity of the test material. Testing laboratory: Industrial Hygiene & Toxicology, Standard Oil Company of California, safety division. Report no.: SOCO 31/I:91 non-GLP, Unpublished	Y	Chevron Chemical Company
Section 4.2	Randall D and Robinson E	1978	Acute Toxicologic Evaluation of the test material. Journal of the American College of Toxicology 1(1):72-73. Non-GLP. Published	N	Monsanto Company-Dept. of Med. & Health Sciences
Section 4.4	Waid, Dougherty & Wong	1989	The four-hour skin irritation potential of the test material. Testing laboratory: Chevron Environmental Health Center, Inc. Report no.: CEHC 2932. GLP, Unpublished.	Y	Chevron Research Company, Oronite Technology Division,
Section 4.4	Cavalli, Hallesy and Spence	1968	Toxicity of the test material. Testing laboratory: Industrial Hygiene & Toxicology, Standard Oil Company of California, safety division. Report no.: SOCO 31/I:91 Non-GLP, Unpublished	Y	Chevron Chemical Company
Section 4.4	Mürmann, P	1984b	Test of the Acute Skin Irritant Effect of the test material. Testing laboratory: Hüls Company Ltd. Report no.: Final Report No. 0287. Non-GLP, Unpublished	Y	American Chemistry Council, Petroleum Additives Panel - HERTG Consortium
Section 4.4	Anon	2006	SIDS Initial Assessment Report for SIAM 22 Non-GLP, published	N	American Chemistry Council, Petroleum Additives Panel - HERTG Consortium
Section 4.4	Mürmann, P	1984c	Test of the Acute Irritant Effect on Eyes and Mucosa of the test material. Testing laboratory: Hüls Company Ltd. Report no.: Final Report No. 0288. Non-GLP, Unpublished	Y	American Chemistry Council, Petroleum Additives Panel - HERTG Consortium
Section 4.4	Mürmann, P	1988	Test on Acute Dermal Irritation Effect of pure test material. Testing laboratory: Hüls Company Ltd. Report	Y	American Chemistry Council,

Data point / Reference number	Author(s)	Year	Title	Data protection claimed	Owner
			no.: Final Report No. 1188 Non-GLP, Unpublished		Petroleum Additives Panel - HERTG Consortium
Section 4.4	Mürmann, P	1991	Test for Acute Dermal Irritation of the test material on rabbits. Testing laboratory: Hüls Company Ltd. Report no.: Final Report No. AH 91/0036. Non-GLP, Unpublished	Y	American Chemistry Council, Petroleum Additives Panel - HERTG Consortium
Section 4.4	Waid, Rogers & Wilkenfeld	1990	The acute eye irritation potential of the test material in adult albino rabbits. Testing laboratory: Chevron Environmental Health Center, Inc. Report no.: CEHC 3191 GLP, Unpublished	Y	Chevron Research Company, Oronite Technology Division,
Section 4.4	Cavalli, Hallesy and Spence	1968	Toxicity of the test material. Testing laboratory: Industrial Hygiene & Toxicology, Standard Oil Company of California, safety division. Report no.: SOCO 31/I:91 Non-GLP, Unpublished	Y	Chevron Chemical Company
Section 4.6	Morris	1997	Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Technique). Testing laboratory: Hill Top Research, Ltd., Miarniville, Ohio. Report no.: 96-8243-21. Study number: 96-45. GLP. Unpublished	Y	Chevron Research and Technology Company
Section 4.6	Silveira et al	1983	Modified Buehler test for the skin sensitisation potential of the test material. Testing laboratory: Chevron Environmental Health Center, Richmond, California. Report no.: SOCAL 1983. GLP. Unpublished	Y	Chevron Chemical Company, Oronite Additives Department
Section 4.7	Harriman, JF	2004	A 14-Day Oral (Gavage) Study of Tetrapropenyl Phenol (CAS RN 74499-35-7) in Rats Testing laboratory: IL Research Laboratories, Inc., 1407 George Road, Ashland, Report OHWIL-186031 GLP. Unpublished	Y	American Chemistry Council, Petroleum Additives Panel, Health Environmental and Regulatory Task Group
Section 4.7	Harriman, JF	2004	A 28-day oral (gavage) toxicity study of the test material (cas rn 74499-35-7) in rats (with functional observational battery and motor activity determinations).	Y	American Chemistry Council, Petroleum Additives Panel,

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			Testing laboratory: WIL Research Laboratories, Inc., Ashland, Ohio. Report no.: WIL-186032. GLP. Unpublished		Health Environmental and Regulatory Task Group
Section 4.7	Vogin, E	1970a	Subacute feeding studies with the test material in rats and dogs. Testing laboratory: Food and Drug Research Laboratories. Report no.: 0088. Non-GLP. Unpublished	Y	GAF Corporation, Dyestuff and Chemical Division, New York
Section 4.7	Reyna & Thake	1988	Four Week Feeding Study of the test material in Sprague-Dawley Rats. Testing laboratory: Monsanto Agricultural Company, Environmental Health Laboratory, St. Louis, Missouri. Report no.: ML-87-041. GLP. Unpublished	Y	Monsanto Company, St. Louis, Missouri
Section 4.7	Haas MC	2011	A 90-Day Dietary Dose Range-Finding Toxicity Study of Tetrapropenyl Phenol in Rats Testing laboratory: WIL Research Laboratories, LLC, 1407 George Road, Ashland, OH Report no.: WIL-186054 GLP. Unpublished	Y	The American Chemistry Council
Section 4.7	Vogin, E	1970b	Sub-acute feeding studies with the test material in rats and dogs. Testing laboratory: Food and Drug Research Laboratories. Report no.: 0091. Non-GLP. Unpublished	Y	GAF Corporation, Dyestuff and Chemical Division, New York
Section 4.9	Machado et al	1989	MICROBIAL/MAMMALIAN MICROSOME PLATE INCORPORATION MUTAGENICITY ASSAY WITH THE TEST MATERIAL GLP. Unpublished	Y	Chevron Research Company, Products Research Department
Section 4.9	Schörberl, P	1992b	Determining the Mutagenicity of the test material in a Salmonella/Mammal Microsome Mutagenicity Test Following the Ames Mutagenicity Test, Following ECC Guideline 84/449, B14. Report no.: Final Report Am 92/7. GLP. Unpublished	Y	Chevron Research Company, Products Research Department
Section 4.9	Condray	1987	Microbial/mammalian microsome plate incorporation mutagenicity assay with the test material. Testing laboratory: Chevron Environmental Health Center, Inc., Richmond, California.	Y	Chevron Research Company, Products Research Department

Data point / Reference number	Author(s)	Year	Title	Data protection claimed	Owner
			Report no.: CEHC 2933. GLP. Unpublished		
Section 4.9	Condray	1987	Microbial/mammalian microsome plate incorporation mutagenicity assay with the test material. Testing laboratory: Chevron Environmental Health Center, Inc., Richmond, California. Report no.: CEHC 2933. Unpublished	Y	Chevron Research Company, Products Research Department
Section 4.11	Edwards, TL	2010	A dietary two-generation reproductive toxicity study of the test material in rats (Draft). Testing laboratory: WIL Research Laboratories, Inc., Ashland, Ohio. Report no.: WIL-186053. GLP, Unpublished	Y	American Chemistry Council, Petroleum Additives Panel, Health Environmental and Regulatory Task Group
Section 4.11	Knapp, JF	2006	An oral (gavage) one-generation reproductive toxicity study of the test material in rats. Testing laboratory: WIL Research Laboratories, Inc., Ashland, Ohio. Report no.: WIL-186033. GLP, Unpublished	Y	American Chemistry Council, Petroleum Additives Panel, Health Environmental and Regulatory Task Group
Section 4.11	Schroeder, R	1985	A range-finding study to evaluate the toxicity of the test material in the pregnant rat. Testing laboratory: Bio/dynamics, Inc, East Millstone, New Jersey 08873. Report no.: 84-2882. GLP, Unpublished	Y	Monsanto Company, St. Louis, Missouri
Section 4.11	Schroeder, R	1987	A teratology study in rats with the test material. Testing laboratory: Bio/dynamics, Inc, East Millstone, New Jersey Report no.: 84-2883 GLP, Unpublished	Y	Monsanto Company, St. Louis, Missouri
Section 5.1.2	Mead & McKenzie	2005	Assessment of inherent biodegradability; Concawe test. Testing laboratory: Safepharm Laboratories Limited. Report no.: 1666/040 GLP, unpublished	Y	American Chemistry Council
Section 5.1.2	Schörberl, P	1992a	Bestimmung der biologischen Abbaubarkeit von Dodecylphenol T im Modifizierten OECD-Sturm-Test. Testing laboratory: Huels AG. Report no.: ST-54/92.	Y	Sasol Germany GmbH.

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			GLP, unpublished		
Section 5.2.1	Woolley & O'Connor	2010	Determination of physico-chemical properties, Harlan Laboratories Ltd, Shardlow, Report No.: 3157/0001. GLP, Unpublished	Y	Calicio Ltd. On behalf of Additives Technical Committee, Task Force 33C
Section 5.3	A. Blankinship, T. Z. Kendall & H. O. Krueger	2006	A Bioconcentration Test with the Rainbow Trout (*Oncorhynchus mykiss*). Testing laboratory: Wildlife International, Ltd., Easton, Maryland. Report no.: 264A-107. GLP, unpublished	Y	American Chemistry Council's Risk Assessment Task Group
Section 5.4.1	Boeri, Ward & Kowalski	1994	Acute Toxicity of the Water Accommodated Fractions (WAFs) of the test material to the Fathead Minnow, *Pimephales promelas*. Testing laboratory: T. R. Wilbury Laboratories, Inc., Marblehead, Massachusetts. Report no.: 74-CM-618. GLP, upublished	Y	Chemical Manufacturers Association
Section 5.4.1	Ward, Kowalski & Boeri	1994	Acute Toxicity of the test material to the Fathead Minnow, Pimephales promelas-Dispersion method. Testing laboratory: T. R. Wilbury Laboratories, Inc., 40 Doaks Lane, Marblehead, Massachusetts 01945. Report no.: 75-CM-618. GLP, unpublished	Y	Chemical Manufacturers Association
Section 5.4.1	Griffen & Thompson	1981	Acute Toxicity of the test material to the Fathead Minnow, *Pimephales promelas*. Testing laboratory: ABC Laboratories Inc. Report no.: #27038 GLP, unpublished	Y	Monsato Industrial Chemicals Company
Section 5.4.1	Scholz, N	1993	Determining the Acute Effect of the test material on Fish (Following EC Guideline 84/449 C1). Report no.: Final Report F1224. GLP, unpublished	Y	American Chemistry Council, Petroleum Additives Panel - HERTG Consortium
Section 5.4.2	Sewell & McKenzie	2005a	Acute toxicity to daphnia magna. Testing laboratory: Safepharm Laboratories Limited, Shardlow Report no.: 1666/027. GLP, unpublished	Y	American Chemistry Council

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Part B, Section 3	Ward & Magazu	1994	Acute Toxicity of the Water Accommodated Fractions (WAFS) of the test material to the Daphnid, Daphnia magna. Testing laboratory: T. R. Wilbury Laboratories, Inc., Marblehead, Massachusetts . Report no.: 72-CM-618 GLP, unpublished	Y	Chemical Manufacturers Association
Section 5.4.2	Calvert & Adams	1981	Acute toxicity of several alkylphenols and orthochlorophenol to daphnia magna. Testing laboratory: MIC Environmental Sciences. Report no.: ES-81-SS-19. GLP, unpublished	Y	Monsanto Industrial Chemicals Company, Environmental Sciences Section
Section 5.4.2	Scholz, N	1992a	Determining the Effects of the test material on the Swimming Behaviour of Daphnia Magna (Following EC Guideline 84/449, November 1989). Report no.: Final Report DK 505. Unpublished	Y	American Chemistry Council, Petroleum Additives Panel - HERTG Consortium
Section 5.4.2	Simon, K	1998	Static renewal acute toxicity of the test material to the mysid shrimp, Mysidopsis bahia. Water Accommodated Fraction Study. Testing laboratory: EnviroSystems, Incorporated, Hampton, New Hampshire. Report No: GLP, Unpublished	Y	American Chemistry Council, Petroleum Additives Panel - HERTG Consortium
Section 5.4.2	Sewell & McKenzie	2005b	Daphnia magna reproduction test. Testing laboratory: Safepharm Laboratories Limited, Shardlow Report no.: 1666/028 GLP, Unpublished	Y	American Chemistry Council
Section 5.4.3	Vryenhoef & McKenzie	2005	Algal inhibition test. Testing laboratory: Safepharm Laboratories Limited, Shardlow Report no.: 1666/076 GLP, Unpublished	Y	American Chemistry Council
Section 5.4.3	Ward, Boeri & Magazu	1994	Acute Toxicity of the Water Accommodated Fractions (WAFS) of the test material to the Freshwater Alga, Selenastrum capricornutum. Testing laboratory: T. R. Wilbury Laboratories, Inc. Marblehead, Massachusetts Report no.: 73-CM-618 GLP, Unpublished	Y	Chemical Manufacturers Association

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Part B, Section 5.4.3	Scholz, N	1992b	Determining the Effects of the test material on the Growth of Scenedesmus Subspicatus 86.81. SAG (Algae Growth Inhibition Test, Following EEC G88/302). Report no.: Final Report AW240. GLP, Unpublished	Y	American Chemistry Council, Petroleum Additives Panel - HERTG Consortium
Section 5.4.1	Scholz, N	1993	Determining the Acute Effect of the test material on Fish (Following EC Guideline 84/449 C1). Report no.: Final Report F1224. GLP, Unpublished	Y	American Chemistry Council, Petroleum Additives Panel - HERTG Consortium