Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

biphenyl-2-ol; 2-phenylphenol; 2-hydroxybiphenyl

EC Number: 201-993-5
CAS Number: 90-43-7

CLH-O-0000007210-88-01/F

Adopted

1 December 2022

February 2023: latest version, replacing all previous versions

1 December 2022

CLH-O-0000007210-88-01/F

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

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

Chemical name: **biphenyl-2-ol; 2-phenylphenol; 2-hydroxybiphenyl**

EC Number: **201-993-5**

CAS Number: **90-43-7**

The proposal was submitted by Spain and received by RAC on **21 February 2022.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Spain has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **28 February 2022**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **29 April 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Miguel A. Sogorb**

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **1 December 2022** by **consensus**.

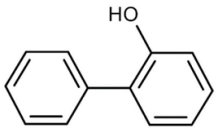
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	604-020-00-6	biphenyl-2-ol; 2-phenylphenol; 2-hydroxybiphenyl	201-993-5	90-43-7	Skin Irrit. 2 Eye Irrit. 2 STOT SE 3 Aquatic Acute 1	H315 H319 H335 H400	GHS07 GHS09 Wng	H315 H319 H335 H400			
Dossier submitters proposal	604-020-00-6	biphenyl-2-ol; 2-phenylphenol; 2-hydroxybiphenyl	201-993-5	90-43-7	**Retain** Aquatic Acute 1 **Add** Carc. 2 Aquatic Chronic 1 **Modify** Skin Corr. 1 Eye Dam. 1 **Remove** STOT SE 3	**Retain** H400 **Add** H351 H410 **Modify** H314 H318 **Remove** H335	**Retain** GHS09 **Add** GHS08 GHS05 **Modify** Dgr **Remove** GHS07	**Add** H351 H410 **Modify** H314 **Remove** H335 H400		**Add** M = 1 M = 1	
RAC opinion	604-020-00-6	biphenyl-2-ol; 2-phenylphenol; 2-hydroxybiphenyl	201-993-5	90-43-7	**Retain** Aquatic Acute 1 **Add** Carc. 2 Skin Sens. 1B Aquatic Chronic 1 **Modify** Skin Corr. 1 Eye Dam. 1 **Remove** STOT SE 3	**Retain** H400 **Add** H351 H317 H410 **Modify** H314 H318 **Remove** H335	**Retain** GHS09 GHS07 **Add** GHS08 GHS05 **Modify** Dgr	**Add** H351 H317 **Modify** H314 **Remove** H335 H400		**Add** M = 1 M = 1	
Resulting Annex VI entry if agreed by COM	604-020-00-6	biphenyl-2-ol; 2-phenylphenol; 2-hydroxybiphenyl	201-993-5	90-43-7	Carc. 2 Skin Corr. 1 Eye Dam. 1 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H351 H314 H318 H317 H400 H410	GHS08 GHS05 GHS07 GHS09 Dgr	H351 H314 H317 H410		M = 1 M = 1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Biphenyl-2-ol, or 2-phenylphenol, or orto-phenylphenol (OPP), is under renewal as a plant protection product under Regulation (EC) N° 1107/2009, and it has been reviewed for use as a biocide under the Biocidal Products Regulation (EU) No 528/2012. It has the chemical structure shown below:



OPP has a current Annex VI entry of Regulation (EC) No.1272/2008 as Skin Irrit. 2 (H315: causes skin irritation); Eye Irrit. 2 (H319: causes serious eye irritation); STOT SE 3 (H335: may cause respiratory irritation) and Aquatic Acute 1 (H400: very toxic to aquatic life).

During the consultation, one Member State Competent Authority (MSCA) released a general comment asking why sodium salt biphenyl-2-olate (CAS 132-27-4) is listed in section 3.6 as "parent substance" while it is neither included in the identity under section 1.3 nor considered for the physical and chemical properties in section 2.2.2. The dossier submitter (DS) replied that the sodium salt is covered for the renewal of the active substance according to plant protection product regulations but is not addressed for the harmonised classification and labelling regulation.

During the consultation, a company-manufacturer submitted a large and detailed set of comments focused on endocrine disruption. This set of comments was extensively replied to by the DS. RAC notes that endocrine disruption is not a hazard class defined in the Regulation (EC) N° 1272/2008 and therefore these comments are not at present relevant for classification purposes.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The DS proposed no classification for all physical hazards.

OPP is a solid and therefore hazard classes for gases and liquids are not applicable. Biphenyl-2-ol does not contain the bivalent O-O group and is thus not an organic peroxide.

Explosives – no classification

No experimental data were available. OPP contains neither oxidising groups nor other chemically unstable functional groups. Thus, it is incapable of rapid decomposition with the evolution of gases or release of heat *i.e.*, the solid material does not present any risk for explosion.

Flammable solids – no classification

In an EC A.10 study (B.2.9/01), OPP did not ignite on contact with the ignition source. Therefore, the classification criteria were not met.

Self-reactive substances – no classification

No test data were available. A self-reactive substance corresponds to a thermally unstable solid liable to undergo a strong exothermic decomposition even without participation of oxygen (air).

OPP is an organic compound that has a melting point of 56.7ºC. According to Tables A6.1 and A6.2 of the UN Recommendations on Transport of Dangerous Goods (RTDG), Manual of Tests and Criteria, it does not contain any functional groups that are associated with explosive or self-reactive properties.

Pyrophoric solids – no classification

No data were available using test N.2 in part III, subsection 33.3.1.4 of the UN RTDG, Manual of Tests and Criteria. OPP does not deliver indications of pyrophoric properties during the realization of tests as defined in EC-A.10 and EC-A.12 (B.2.9/01). Furthermore, OPP does not ignite spontaneously in contact with air based on experience of handling and use.

According to Section 2.10.4.1 of Annex 1 of CLP, the classification procedure for pyrophoric solids need not be applied when experience in manufacture and handling shows that the substance does not spontaneously ignite upon coming into contact with air at normal temperatures. There are no reports in the available studies of OPP spontaneously igniting when in contact with air. Therefore, OPP does not meet the criteria for classification as a pyrophoric solid.

Self-heating substances – no classification

The result of a UN Test N.4 (B.2.9/02) was negative with a 100 mm sample cube at 140 °C. OPP is not self-heating.

Substances which in contact with water emit flammable gases – no classification

Section 2.12.4.1 of Annex I of CLP states that the classification procedure for this hazard class need not be applied if the chemical structure of the substance or mixture does not contain metals or metalloids, or if experience in production or handling shows that the substance does not react with water, or if the substance is known to be soluble in water to form a stable mixture. According to the mentioned criteria classification for this hazard class is not applicable to OPP.

Oxidising solids – no classification

Based on scientific judgement it is certified that due to the structural formula, OPP does not contain oxidising groups in its molecular backbone and thus may not react exothermically with a combustible material.

According to Section 2.14.4.1-point (b) of Annex I of CLP, for organic substances the classification procedure for this hazard class shall not apply if the substance or mixture contains oxygen and this element is chemically bound only to carbon or hydrogen. OPP contains an oxygen atom that is chemically bound only to carbon or hydrogen and therefore, it fulfils the criteria for no classification as an oxidising solid.

Corrosive to metals – no classification

No test data was available. According to the ECHA Guidance on the Application of the CLP Criteria (version 5.0 July 2017), the UN Test C.1 excludes solids while it considers "solids that may become liquid upon transportation". OPP is supplied as a dry solid and its measured melting point is > 55ºC, which is the test temperature required in the UN Test C.1. Furthermore, evidence from manufacture and handling shows that OPP is not corrosive to metals. Therefore, the substance does not meet the criteria for classification as corrosive to metals.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC agrees with the DS not to classify OPP (OPP) for physical hazards.

Organic peroxides

RAC agrees with the DS's conclusion that, because OPP does not contain the bivalent O-O group, it is not an organic peroxide.

Explosives

RAC agrees with the DS's conclusion not to classify the substance as an explosive. OPP does not contain any chemical groups or structural features associated with explosive properties as specified in Table A6.1 in Appendix 6 of the UN Recommendations on Transport of Dangerous Goods (RTDG), Manual of Tests and Criteria. Therefore, OPP does not meet the criteria for classification as an explosive substance (CLP 2.1.4.3 (a)).

Flammable solids

The screening tests required in the CLP Criteria (CLP 2.7.2.1, UN-MTC 33.2.1) were not available. However, OPP did not ignite on contact with the ignition source in an EC A.10 study (B.2.9/01) indicating that the substance was not a flammable solid.

Therefore, RAC concludes no classification is warranted.

Self-reactive substances

RAC agrees with the DS's conclusion that the classification criteria were not met. This is based on there being no chemical groups or structural features present in the molecule associated with explosive or self-reactive properties, as specified in Table A6.1 and A6.3 in Appendix 6 of the UN Recommendations on Transport of Dangerous Goods (TDG), Manual of tests and Criteria (CLP 2.8.4.2 (a)).

Pyrophoric solids

RAC agrees with the DS's conclusion not to classify OPP. Experience in manufacture and handling shows that the substance does not ignite spontaneously on coming into contact with air at normal temperatures (CLP 2.10.4.1).

Self-heating substances

RAC agrees with the DS that no classification is warranted. The result of a UN Test N.4 (B.2.9/02) was negative with a 100 mm sample cube at 140 °C (CLP Figure 2.11.1).

Substances which in contact with water emit flammable gases

RAC agrees with the DS's conclusion that classification is not warranted for OPP (CLP 2.12.4.1).

Oxidising solids

RAC agrees with the DS's conclusion that because the substance contains oxygen only chemically bonded to carbon or hydrogen, the classification procedure for this hazard class shall not apply (CLP 2.14.4.1).

Corrosive to metals

RAC agrees with the DS to not classify OPP as corrosive to metals based on the proven experience (CLP 2.16.4.2). The UN Test C.1 is not applicable because it excludes solids. OPP is supplied as

a dry solid and its measured melting point is greater than the test temperature used in the UN Test C.1 test (55ºC).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

DS proposed no classification of OPP for acute toxicity based on studies performed according to the corresponding OECD TG and GLP compliant. The study results yielded a LD_{50} of 2733 mg/kg bw by oral route; a LD_{50} higher than 2000 mg/kg bw by dermal route and a LC_{50} higher than 0.036 mg/L (maximum attainable concentration) by inhalation route. In all cases, these results were supported by the less reliable studies.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The table below summarises the results of the acute oral toxicity studies with animals. Only one of available studies (B.6.2.1-05) clearly complies with OECD TG and GLP resulting in a LD_{50} of 2733 mg/kg bw for male and female rats. The other five studies (three in rats and two in mice) presented significant deficiencies (see table below for details) and therefore these studies are considered as supportive. The results of the three supportive studies in rat (B.6.2.1-01, B.6.2.1-02 and B.6.2.1-03) are in line with the key study, with LD_{50} values greater than 2000 mg/kg bw. One of the two supportive studies in mice (B.6.2.1-04) resulted in a LD_{50} of 1050 mg/kg bw for females and 1200 mg/kg bw for males suggesting higher sensitivity of mouse than in rat. However, it was not confirmed in the second study (B.6.2.1-06) with a different strain of mice, where LD_{50} higher than 3000 mg/kg bw were reported for both males and females.

***Table**: Summary of animal studies on acute oral toxicity with OPP*

Study	Dose level	Results	Reference
Acute oral toxicity study in rats OECD TG 401 (1987) GLP: Yes Fischer 344 rats 5 animals/sex/dose **Key study**	Purity: 99.9% Vehicle: corn oil Gavage 500, 2500 and 5000 mg/kg bw 14-day observation period	Mortalities males: 0/5, 2/5 and 5/5 for 500, 2500 and 5000 mg/kg bw; respectively. Mortalities females: 0/5, 2/5 and 5/5 for 500, 2500 and 5000 mg/kg bw; respectively. Clinical signs: observed at 2500 and 5000 mg/kg bw: lacrimation, salivation, chromorhinorrhea, laboured respiration, decreased activity, lateral recumbence and urine and faecal soiling in the perineal area. Body weight: all surviving animals gained weight during the observation period.	B.6.2.1-05, 1994

Study	Dose level	Results	Reference
		Necropsy at 2500 mg/kg bw: haemolysed blood in the digestive tract (dead on day 2) and perineal soiling (dead on day 3); fibrous adhesions between the serosa of the non-glandular portion of the stomach and liver in surviving males. Necropsy at 5000 mg/kg bw: all animals dead on day 1 (5 females, 2 males) had no gross lesions; animals dead on day 2 haemolysed blood in the digestive tract; animals dead on day 3 perineal soiling and lung congestion lesions. **LD_{50} = 2733 mg/kg bw (both sexes)**	
Acute oral toxicity study in rats Prior to OECD TG 401 GLP: Not applicable Male Wistar rats 10 rats/group Supportive study	Purity: not indicated Vehicle: polyethylene glycol Dosing into duodenum 1500, 2000, 3100, 4000, 4500 and 5000 mg/kg bw 14-day observation	Mortalities: 0/10, 4/10, 4/10, 6/10, 8/10 and 10/10 for 1500, 2000, 3100, 4000, 4500 and 5000 mg/kg bw; respectively. Clinical signs: anaesthesia, impaired general condition, abdominal recumbence, lateral recumbence. No macroscopic findings Deviations: test material no characterised; animals non-fasted; dosing into duodenum; random necropsies; individual body weights not reported. **LD_{50} = 2980 mg/kg bw (male rats)**	B.6.2.1-01, 1981
Acute oral toxicity study in rats Prior to OECD TG 401 GLP: Not applicable 15 male rats/group Strain: not indicated Supportive study	Purity: not indicated Vehicle: polyethylene glycol Gavage 500, 1000 and 2500 mg/kg bw 7-day observation	No mortalities No clinical signs Deviations: only brief summary in German; strain and weight of animals not reported; animals non-fasted; 7 days observation period; no necropsy. **LD_{50} > 2500 mg/kg bw**	B.6.2.1-02, 1969
Acute oral toxicity study in rats Prior to OECD TG 401 GLP: Not applicable 10-20 male rats/group Strain: not indicated Supportive study	Purity >98% Vehicle: olive oil/gum acacia Gavage 1600, 2000, 2400, 2800, 3000, 3200 and 4000 mg/kg bw	Mortalities: 0/10, 4/20, 5/20, 8/10, 6/10, 6/10 and 16/19 for 1600, 2000, 2400, 2800, 3000, 3200 and 4000 mg/kg bw; respectively. Clinical signs: not observed Necropsy: not performed Deficiencies: only brief summary; unknown batch and strain of animals; incomplete method description; individual body weights only recorded at the beginning; no necropsy. **LD_{50} = 2700 mg/kg bw**	Hodge *et al.* 1952 B.6.2.1-03 Published study

Study	Dose level	Results	Reference
	14-day observation period		
Acute oral toxicity study in mice GLP: Not applicable ddY mice 10 mice/sex/group Supportive study	Purity not reported Vehicle: propylene glycol Gavage 0, 414, 538, 700, 910, 1183, 1538 and 2000 mg/kg bw 14-day observation	Mortalities males: 0/10, 0/10, 0/10, 1/10, 0/10, 5/10, 7/10 and 10/10 for 0, 414, 538, 700, 810, 1183, 1538 and 2000 mg/kg bw; respectively. Mortalities females: 0/10, 0/10, 0/10, 0/10, 0/10, 6/10, 10/10 and 10/10 for 0, 414, 538, 700, 810, 1183, 1538 and 2000 mg/kg bw; respectively. Clinical signs: decrease of spontaneous movement, limb position, staggering gait and low respiratory rate were the main clinical symptoms. Body weight: body weight gain and final body weights were depressed in all treated males. Final body weights of surviving females did not differ from control. Deviations: publication in Japanese (only abstract and results table/graphs in English); not possible to check the method. **LD_{50} males = 1200 mg/kg bw** **LD_{50} females = 1050 mg/kg bw**	Taniguchi *et al.* 1981 B.6.2.1-04 Published study
Acute oral toxicity study in mice GLP: Not applicable IRC mice 10 mice/sex/group Supportive study	Purity: 98% Vehicle: olive oil Oral Single dose 1000, 1500, 2250, 3375, 5063 and 7594 mg/kg bw 14-day observation	Mortalities males: 0/10, 0/10, 0/10, 2/10, 4/10, 8/10 and 10/10 for 0, 1000, 1500, 2250, 3375, 5063 and 7594 mg/kg bw; respectively. Mortalities females: 0/10, 0/10, 0/10, 3/10, 7/10, 8/10 and 9/10 for 0, 1000, 1500, 2250, 3375, 5063 and 7594 mg/kg bw; respectively. Clinical signs: decrease of motor activity, sedation and lacrimation. Deficiencies: publication in Japanese (only abstract and results table/graphs in English); not possible to check the method. **LD_{50} males = 3499 mg/kg bw** **LD_{50} females = 3152 mg/kg bw**	Tayama *et al.* 1983 B.6.2.1-06 Published study

The table below summarises the results of the dermal acute toxicity studies. One of these two studies (B.6.2.2-01) complies with OECD TG 402 with no deviations. The resulting LD_{50} is > 2000 mg/kg bw for male and female rats. The other study (B.6.2.2-02) is considered as supportive, since only 2 animals per sex were used. The resulting LD_{50} (> 50000 mg/kg bw) is in line with the first study findings.

***Table**: Summary of animal studies on acute dermal toxicity with OPP*

Study	Dose level	Results	Reference
Acute dermal toxicity study in rats OECD TG 402 (1987) GLP: Yes Wistar rats 5 rats/sex **Key study**	Purity: 99.89% Vehicle: Cremophor E 2000 mg/kg bw 24h exposure 14-day observation period	No mortalities Clinical signs: slight reddening at the application site on the day 1 in both male and female rats. On day 5 it turned to incrustation although symptoms reversed by day 14. Body weight: slight decrease in body weight in 3 females during the first week. Necropsy: no treatment-related effects. **LD_{50} > 2000 mg/kg bw (both sexes)**	B.6.2.2-01, 1991
Acute dermal toxicity study in rabbits OECD TG 402 (1981) GLP: No New Zealand White (NZW) rabbits 2 rabbits/sex Supportive study	Purity: 99.73% Applied dry on the skin. Water was added to simulate moistened skin 5000 mg/kg bw 24h exposure 14-day observation period	No mortalities Clinical signs: lethargy following treatment. Moderate erythema and oedema and marked necrosis at the application site. Body weight: one female showed a decrease at the end of the study. Deviations: only 2 animals per sex **LD_{50} > 5000 mg/kg bw (both sexes)**	B.6.2.2-02, 1981

The table below summarises the results of the acute inhalation toxicity studies with animals. One of the three studies (B.6.2.3-01) complies with OECD TG 403 with no deviations. The resulting LC_{50} is > 0.036 mg/L (maximum attainable concentration) for male and female rats. The other two studies are considered supportive only, due to deviations from the method, where the atmosphere was not characterized in any of the studies, and the exposure times were of 1 hour (B.6.2.3-02) or 7 hours (B.6.2.3-02) instead of 4 hours.

***Table**: Summary of animal studies on acute inhalation toxicity with OPP*

Study	Dose level	Results	Reference
Acute inhalation toxicity study in rats OECD TG 403 (1981) GLP: Yes Fischer 344 rats 5 rats/sex	Purity: 99.8% Nominal concentration: 13.00 mg/L Mean max. attainable Concentration: 0.036 mg/L	No mortalities Clinical signs: 2 males and 3 females had general soiling and one female had perineal soiling following exposure. All animals appeared normal on the day after exposure. All rats gained weight during observation period No abnormalities noted in necropsy	B.6.2.3-01, 1992

Study	Dose level	Results	Reference
Key study	Particles < 1 µm: > 50% MMAD and GSD were not calculated because the particle size distribution was not log-normal. Observation period: 14 days 4-h exposure (nose only)	**LC_{50} >0.036 mg/L/4 h**	
Acute inhalation toxicity study in rats Prior to OECD TG 403 (1981) GLP: No Wistar II rats 20 male rats/group Supportive study	Purity: not stated 0.228, 0.447 and 0.949 mg/L 1h exposure Observation period 7 days	Deviations: test substance and test atmosphere not characterized, exposure time only 1h, observation period only 7 days. **LC_{50} >0.949 mg/L/1 h**	B.6.2.3-02, 1977
Acute inhalation toxicity study in rats Time-saturation test No guideline (similar to OECD TG 403, 1981) Annex: Inhalation hazard test Wistar rats 5 rats/sex Supportive study	Purity: >99.5% Doses: not determined (air enriched with vapour of OPP) Exposure: 7-h (whole body)	Deviations: 7h exposure, batch and test article preparation not reported, concentration of the test substance not measured, information of exposure parameters and individual body weights were not reported. LC_{50}: Not determined	B.6.2.3-03, 1982

Comparison with the criteria

The most reliable acute oral toxicity study showed a LD_{50} for OPP of 2733 mg/kg bw. This rat study was in line with several supportive studies. In a study published in 1981 (B.6.2.1-04), mice resulted more sensitive than rats, however this was not confirmed in a second supportive study published in 1983 (B.6.2.1-06). In conclusion, the LD_{50} is above the threshold value of 2000 mg/kg bw for triggering classification and **RAC supports the DS's proposal for no classification of OPP for acute oral toxicity**.

The most reliable acute dermal toxicity study in rats showed a LD_{50} higher than 2000 mg/kg bw. The acute dermal toxicity study in rabbits showed a LD_{50} higher than 5000 mg/kg bw. In conclusion, the LD_{50} of OPP is above the threshold value of 2000 mg/kg bw for triggering

classification and **RAC supports the DS's proposal for no classification of OPP for acute dermal toxicity**.

OPP at the maximum attainable concentration (0.036 mg/L) caused no mortalities. Consequently, **RAC supports the DS's proposal for no classification of OPP for acute inhalation toxicity**.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

DS proposed no classification for STOT SE because the only effects noted in a reliable study were detected at concentrations causing mortality and above the threshold for triggering classification. DS proposed removing the current classification as STOT SE 3 for respiratory tract irritation since such effects were not observed in the acute inhalation toxicity studies and that according to "Guidance on the Application of the CLP Criteria" a classification for STOT SE 3 in a corrosive substance is considered superfluous.

Comments received during consultation

One MSCA questioned the removal of the STOT SE 3 classification given the corrosive nature of OPP. The DS replied that OPP data on humans does not provide any evidence of respiratory irritation and no clinical or necropsy findings in animals as evidence respiratory tract irritation. Moreover, the CLP guidance[1] states that corrosivity is considered to implicitly cover the potential to cause respiratory tract irritation and therefore the STOT SE 3 classification would be superfluous.

Assessment and comparison with the classification criteria

Studies in rats

In the acute oral toxicity studies carried out by treating rats with OPP, several clinical signs were observed. In two studies, considered as supportive, anaesthesia, impaired general condition, abdominal recumbence and lateral recumbence were reported from 1500 mg/kg bw (B.6.2.1-01) and progressive depression from 2000 mg/kg bw (B.6.2.1-03). In the single fully acceptable acute toxicity study with OPP (B.6.2.1-05) lacrimation, salivation, chromorhinorrhea, laboured respiration, decreased activity, lateral recumbence and urine and faecal soiling in the perineal area were observed in both sexes from 2500 mg/kg bw. Necropsy findings occurred also from this dose (haemolysed blood in the digestive tract, perineal soiling, fibrous adhesions between the serosa of the non-glandular portion of the stomach and liver).

A developmental toxicity test in rats (B.6.6.2-01) (see section for reproductive toxicity) reported ataxia for several hours in dams from 300 mg/kg bw/day. The severity of this ataxia was dose-dependent. This last study was also considered as supportive only.

[1] Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, 2017

Studies in mice (all considered as supportive)

In the acute oral toxicity studies in mice treated with OPP, decrease of spontaneous movement, limb position, staggering gait and low respiratory rate (B.6.2.1-04) and decrease of motor activity, sedation and lacrimation (B.6.2.1-06) were the main clinical symptoms.

In a Comet assay with OPP, apathy, semi-anaesthetised state, roughened fur, pallor, staggering gait, sternal recumbence, spasm, shivering, languor, wide-legged gait and slitted eyes were observed at 2000 mg/kg bw (B.6.4.2.2-01) (see section for germ cell mutagenicity).

Comparison with the criteria

STOT SE 1 and 2

STOT SE Categories 1 and 2 are assigned based on findings of significant or severe toxicity. None of the effects observed in acute oral toxicity studies were considered as sufficiently significant or severe as to be taken into account to assign a STOT SE Category 1 or 2. In any case, the only effects observed in a fully acceptable study (B.6.2.1-05) were found in rats treated with OPP in a lethal amount above the range for classification on these categories (2000 mg/kg bw) and close to LD_{50}.

STOT SE 3

STOT SE 3 includes narcotic effects and respiratory tract irritation. Some narcotic effects were observed after administration of OPP:

- in a supportive acute oral study in rats (B.6.2.1-01);
- in a supportive acute oral study in rats (B.6.2.1-03);
- in the acceptable acute oral study in rats (B.6.2.1-05);
- in a supportive developmental toxicity test in rats (B.6.6.2-01);
- in a supportive acute oral study in mice (B.6.2.1-04);
- in a supportive acute oral study in mice (B.6.2.1-06);
- in a supportive Comet assay in mice (B.6.4.2.2-01) at the lethal dose of 2000 mg/kg bw.

About these effects, it could be taken into account that:

- most of the clinical symptoms were observed in supportive studies in which the essential information related to time of onset of symptoms, their reversibility or individual data was not detailed.
- the observed effects in the single acceptable acute oral exposure test were found close to the LD50. These effects should be considered as covered by the adopted oral acute toxicity classification.

Therefore, the above-mentioned narcotic effect cannot warrant a classification of OPP for STOT SE 3.

It is remarkable that no respiratory tract irritation was noted in the acute inhalation toxicity studies, although it could be explained based on the low concentration tested. The current entry in Annex VI of OPP includes STOT SE 3; H335, for respiratory tract irritation. This classification is based on the assumption that because of its proven severe irritation effects, it can be reasonably assumed that OPP is irritating to the airways when inhaled in high concentrations. However, RAC notes that the current CLP guidance states: "*In general, a classification for corrosivity is considered to implicitly cover the potential to cause respiratory tract irritation and so the additional Category 3 is considered to be superfluous*". RAC also notes that no clinical signs or necropsy findings support respiratory tract irritation in animal studies and therefore the removal of STOT SE 3 (H335) complies with the criteria established in the Regulation (EC) No. 1272/2008.

In conclusion, **RAC supports the DS's proposal for no classification of OPP as STOT SE.**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

DS proposed the classification of OPP as Skin Corr. 1; H314, causes severe skin burns and eye damage, based on a skin irritation/corrosion test in rabbits performed following OECD TG 404 and observing GLP. In this study, scars were observed at the end of the 14-day observation period. This proposal was also supported by other less reliable study showing also irreversible skin lesions 7-days after OPP exposure and by the acute dermal toxicity studies.

Comments received during consultation

One MSCA supported the DS's proposal for classification of OPP as skin corrosive.

Assessment and comparison with the classification criteria

The table below summarises the results of the skin corrosion/irritation studies with animals. Two of the six animal studies on skin corrosion/irritation (B.6.2.4-01 and B.6.2.4-06), comply with the guidance test methods, and only one of them is conducted in compliance with GLP (B.6.2.4-01). In the first study (B.6.2.4-01), severe skin effects were observed in four of six animals, with the formation of scars (which are evidence of corrosion). The other acceptable study (B.6.2.4-06), which is prior to GLP, shows slight to moderate irritation effects, which were reversible after 8 days (end of the study). The reason explaining such a difference between these two studies is unclear.

Another four studies (B.6.2.4-02 to B.6.2.4-05) were provided for the assessment of the skin corrosion/irritation of OPP and were considered as supportive studies due to methodological deficiencies. Two of these studies did not even report irritation scores, and the exposure period lasted for 24 hours. In study B.6.2.4-04, the substance showed to be moderately irritating to the skin, and in the study B.6.2.4-05 it showed no irritation effects. The other two supplementary studies did report individual scores. One of them (B.6.2.4-03) showed slight to moderate skin reactions (erythema and/or oedema) which were reversible after 10 days; nevertheless, the exposure period for this study was 30 minutes. In the study B.6.2.4-02 animals were exposed for 4 hours, and five rabbits showed moderate to severe erythema at the 72h observation (grade 4 in 4 animals, and 3 in the other one), which persisted until the end of the study on day 7, showing no reversibility in this period. Likewise, no reversibility could be determined for the oedema (grade 1) present in four animals 72 hours after exposure, which persisted until day 7. It should be noted that in this study, although five rabbits showed severe effects, the erythema (grades 1 and 2) and oedema (grade 1 at 24 h) observed in the other animal, were reversible 72 hours after the exposure.

Two acute dermal toxicity studies are available: one was carried out with rats (B.6.2.2-01) and a slight reddening was observed in the application site on day 1, turned to incrustation on day five, and was reversible by day 14. The other study (B.6.2.2-02) was carried out with four rabbits, and necrosis was observed at the application site in all the treated animals, which could be considered evidence of corrosion.

In addition, to support the evidence that the substance causes irritation to the skin, the local effects observed in the two short-term toxicity studies that applied OPP by dermal route, included hyperkeratosis and acanthosis as a result of an irritant effect in one study (B.6.3.3-01), and ulcerative lesions at the site of application in the other (B.6.3.3-02) (see table in STOT RE section).

***Table**: Summary of the animal studies on skin corrosion/irritation with OPP. E = erythema. O = Oedema.*

Study	Dose level	Results	Reference
Skin irritation/ corrosion in rabbits OECD TG 404 (1987) GLP: Yes NZW rabbits 3 rabbits/sex **Key study**	Purity: 99.9% 0.5 g Applied moistened with 0.3 mL water 4h exposure	See tables below	B.6.2.4-01, 1994

	Male rabbit No					
	0		1		2	
	E	O	E	O	E	O
30 min	1	0	0	0	4#	4
24 h	1	0	1	0	4#	4
48 h	0	0	0	0	4#	4
72 h	0	0	0	0	4#	4
7 d	0	0	0	0	4*	0
15 d	0	0	0	0	4Δ	0
Mean 24/48/72 h	0.3	0.0	0.3	0.0	4.0	4.0
Reversible	Y	-	Y	-	N	Y

	Female rabbit No					
	3		4		5	
	E	O	E	O	E	O
30 min	4#	1	4#	1	4#	2
24 h	4#	1	4#	1	4#	2
48 h	4#	2	4#	0	4#	2
72 h	4#	2	4#	0	4#	2
7 d	4*	0	4*	0	4*	0
15 d	4Δ	0	4Δ	0	4Δ	0
Mean 24/48/72 h	4.0	1.7	4.0	0.3	4.0	2.0
Reversible	N	Y	N	Y	N	Y

Burns observed at application site
* Scabs observed at application site
Δ Scars observed at application site

Study	Dose level	Results	Reference
Skin irritation/ corrosion in rabbits OECD TG 404 GLP: No NZW rabbits 3 rabbits/sex Supportive study	Purity: >99.5% Dose: not described 4h exposure	See tables below	B.6.2.4-02, 1982

	Rabbit No					
	102		101		97	
	E	O	E	O	E	O
2h	1	0	1	1	1	0
24h	0	0	4	2	2	1
48h	4	0	4	2	1	0
72h	4	0	4	1	0	0
7d	4	0	4	1	0	0
Mean 24/48/72h	2.7	0.0	4	1.7	1.0	0.3
Reversible	N	-	N	N	Y	Y

	Rabbit No					
	95		94		83	
	E	O	E	O	E	O
2h	3	0	1	0	4	1
24h	4	2	1	0	4	1
48h	1	4	3	1	4	1
72h	4	1	3	1	4	1
7d	4	1	3	1	4	1
Mean 24/48/72h	3	2.3	2.3	0.7	4	1
Reversible	N	N	N	N	N	N

Deviations: exposure conditions not reported; the study finalised after 7 days (instead of 21 days); first scoring at 2h (instead of 30 min); batch, test article preparation and individual body weights not reported.

<table>
<tr><th>Study</th><th>Dose level</th><th>Results</th><th>Reference</th></tr>
<tr><td>Skin irritation/ corrosion in rabbits

OECD TG 404

GLP: No

NZW rabbits

3 males

Supportive study</td><td>Purity: not indicated

0.5 g

30 min exposure</td><td>
<table>
<tr><td></td><td colspan="6">Rabbit No</td></tr>
<tr><td></td><td colspan="2">100</td><td colspan="2">107</td><td colspan="2">108</td></tr>
<tr><td></td><td>E</td><td>O</td><td>E</td><td>O</td><td>E</td><td>O</td></tr>
<tr><td>1h</td><td>2</td><td>1</td><td>1</td><td>1</td><td>2</td><td>1</td></tr>
<tr><td>24h</td><td>2</td><td>0</td><td>1</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>48h</td><td>1</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>72h</td><td>1</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>10d</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Mean 24/48/72h</td><td>1.3</td><td>0.0</td><td>0.3</td><td>0.0</td><td>1.0</td><td>0.0</td></tr>
<tr><td>Reversible</td><td>Y</td><td>Y</td><td>Y</td><td>Y</td><td>Y</td><td>Y</td></tr>
</table>
Deviations: exposure time 30 min (instead of 4h); reporting deficits: test material not characterised, individual body weights not reported.
</td><td>B.6.2.4-03, 1983</td></tr>
<tr><td>Skin irritation/ corrosion in rabbits

Prior to OECD TG 404

GLP: No

NZW rabbit

1 rabbit/sex

Supportive study</td><td>Purity: not indicated

0.5 g</td><td>The test article was moderately irritating to the skin.

Skin irritation scores were not reported.

No indication of the reversibility of the effects after the 7-day observation period.

Deviations: one page report in German; test material not characterised; application onto the inner surface of the ear; exposure time 24h (instead of 4h); skin irritation scores and individual body weights not reported.</td><td>B.6.2.4-04, 1978</td></tr>
<tr><td>Skin irritation/ corrosion in rabbits and humans

Prior to OECD TG 404

GLP: No

2 rabbits and 11 human volunteers</td><td>0.1% aqueous OPP solution

24h exposure

Application:
- inner side of the ear of the rabbits and
- lower arm of human subjects</td><td>No irritation was observed on the skin of rabbits or human subjects after exposure nor during the 7 days of follow-up.

Skin irritation scores were not reported.

Deviations: brief summary in German; test substance not characterized; aqueous dilutions were used; strain, sex and weight of test animals not reported; application onto the inner surface of the ear; exposure time 24h (instead of 4h); skin irritation scores not reported.</td><td>B.6.2.4-05, 1969</td></tr>
<tr><td>Skin irritation/ corrosion in rabbits

OECD TG 404

GLP: No

NZW rabbits

3 males

Key study</td><td>Purity: 99.5%

0.5 g</td><td>
<table>
<tr><td></td><td colspan="6">Rabbit No</td></tr>
<tr><td></td><td colspan="2">1</td><td colspan="2">2</td><td colspan="2">3</td></tr>
<tr><td></td><td>E</td><td>O</td><td>E</td><td>O</td><td>E</td><td>O</td></tr>
<tr><td>1h</td><td>1</td><td>2</td><td>0</td><td>0</td><td>1</td><td>1</td></tr>
<tr><td>24h</td><td>1</td><td>1</td><td>2</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>48h</td><td>1</td><td>1</td><td>2</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>72h</td><td>1</td><td>1</td><td>2</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>8d</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Mean 24/48/72h</td><td>1.0</td><td>1.0</td><td>2.0</td><td>0.0</td><td>1.0</td><td>0.0</td></tr>
<tr><td>Reversible</td><td>Y</td><td>Y</td><td>Y</td><td>Y</td><td>Y</td><td>Y</td></tr>
</table>
</td><td>B.6.2.4-06, 1981a</td></tr>
</table>

Comparison with the criteria

According to the guideline and GLP compliant study B.6.2.4-01 after an exposure of four hours, 4/6 rabbits showed a mean score per animal of 4.0 for erythema and scar formation in all 4 animals at the end of the study (14 days observation period). According to CLP, classification as skin corrosive is warranted if at least one animal shows a corrosive response (such as scars) at the end of the observation period.

Moreover, according to the CLP guidance if the substance is proven to be corrosive in an acute dermal toxicity test carried out with rabbits with a suitable suspension of solid the classification as skin corrosive applies. An acute dermal toxicity test in rabbits where OPP was applied dry caused necrosis in all of the four rabbits treated.

In summary, according to the available data, OPP fulfils the criteria to classify as corrosive (no subcategorization can be concluded) and **RAC supports the DS's proposal for classification of OPP as Skin Corr. 1; H314, causes severe skin burns and eye damage**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

DS proposed classification of OPP as Eye Dam. 1; H318, causes serious eye damage, based on the classification of the substance as Skin Corr. 1; H314 and the results of an OECD TG 405 study where eye lesions induced by OPP (corneal opacity ≥ 1; conjunctival oedema ≥ 2; corneal opacity ≤ 3 and iris lesions ≤ 1.5) were not reversible after 8 days of observation. Comparable results were also reported in a second study.

Comments received during consultation

A MSCA supported the DS's proposal for classification of OPP as Eye Dam. 1.

Assessment and comparison with the classification criteria

The table below summarises the results of the serious eye damage/irritation studies with animals. Only two of these three studies (B.6.2.5-01 and B.6.2.5-03) are considered acceptable (compliant with the guidance test methods), although the observation periods were too short (8 and 7 days instead of 21) to verify the reversibility of the lesions observed. The last study (B.6.2.5-02) contains only a document of one page in German and is considered as supplementary only.

Table: *Summary of the animal study on serious eye damage/irritation with OPP. E = Erythema; O = Oedema; C = Cornea opacity; I = Iris lesions.*

<table>
<tr><th>Study</th><th>Dose level</th><th>Results</th><th>Reference</th></tr>
<tr><td>Eye irritation

OECD TG 405

GLP: No

Deviations: observation period of 8 days instead of 21.

NZW rabbits

3 males

Key study</td><td>Purity: 99.5%

100 µL

No rinsing</td><td>
<table>
<tr><td></td><td colspan="4">Rabbit 1</td></tr>
<tr><td></td><td></td><td></td><td colspan="2">Conjunctiva</td></tr>
<tr><td></td><td>C</td><td>I</td><td>E</td><td>O</td></tr>
<tr><td>1h</td><td>1</td><td>0</td><td>1</td><td>1</td></tr>
<tr><td>24h</td><td>1</td><td>0</td><td>1</td><td>3</td></tr>
<tr><td>48h</td><td>2</td><td>2</td><td>2</td><td>2</td></tr>
<tr><td>72h</td><td>2</td><td>2</td><td>2</td><td>2</td></tr>
<tr><td>8d</td><td>2</td><td>2</td><td>1</td><td>1</td></tr>
<tr><td>Mean 24/48/72h</td><td>1.67</td><td>1.33</td><td>1.67</td><td>2.33</td></tr>
<tr><td>Revers (72h – 8d)</td><td>N</td><td>N</td><td>Y↓</td><td>Y↓</td></tr>
</table>
<table>
<tr><td></td><td colspan="4">Rabbit 2</td></tr>
<tr><td></td><td></td><td></td><td colspan="2">Conjunctiva</td></tr>
<tr><td></td><td>C</td><td>I</td><td>E</td><td>O</td></tr>
<tr><td>1h</td><td>1</td><td>0</td><td>1</td><td>2</td></tr>
<tr><td>24h</td><td>1</td><td>0</td><td>2</td><td>3</td></tr>
<tr><td>48h</td><td>2</td><td>1</td><td>2</td><td>2</td></tr>
<tr><td>72h</td><td>2</td><td>1</td><td>2</td><td>2</td></tr>
<tr><td>8d</td><td>3</td><td>2</td><td>1</td><td>1</td></tr>
<tr><td>Mean 24/48/72h</td><td>1.67</td><td>0.67</td><td>2.0</td><td>2.33</td></tr>
<tr><td>Revers (72h – 8d)</td><td>N↑</td><td>N↑</td><td>Y↓</td><td>Y↓</td></tr>
</table>
<table>
<tr><td></td><td colspan="4">Rabbit 3</td></tr>
<tr><td></td><td></td><td></td><td colspan="2">Conjunctiva</td></tr>
<tr><td></td><td>C</td><td>I</td><td>E</td><td>O</td></tr>
<tr><td>1h</td><td>2</td><td>0</td><td>1</td><td>2</td></tr>
<tr><td>24h</td><td>2</td><td>0</td><td>1</td><td>2</td></tr>
<tr><td>48h</td><td>2</td><td>1</td><td>1</td><td>2</td></tr>
<tr><td>72h</td><td>2</td><td>1</td><td>2</td><td>2</td></tr>
<tr><td>8d</td><td>2</td><td>2</td><td>1</td><td>1</td></tr>
<tr><td>Mean 24/48/72h</td><td>2.0</td><td>0.67</td><td>1.33</td><td>2.0</td></tr>
<tr><td>Revers (72h – 8d)</td><td>N</td><td>N↑</td><td>Y↓</td><td>Y↓</td></tr>
</table>
N↑ = increased lesion score; N = same score; Y↓ = decreased score</td><td>B.6.2.5-01, 1981b</td></tr>
<tr><td>Eye Irritation

No guidelines

GLP: No

Deviations: purity and batch not reported; no data of ocular lesions score neither any other data was reported

NZW rabbits

1 male and 1 female

Supportive only</td><td>Dose not known</td><td>The test article was strongly irritating and corrosive.

No data on ocular lesions scores nor any other data was reported.</td><td>B.6.2.5-02, 1978</td></tr>
</table>

Study	Dose level	Results	Reference

Eye Irritation

OECD TG 405

GLP: No

Deviations: observation period of 7 days instead of 21.

NZW rabbits

5 males and 1 female

Key study

0.1 g

B.6.2.5-03, 1971

	Rabbit 1			
			Conjunctiva	
	C	I	E	O
24h	1	1	2	3
48h	1	1	3	4
72h	2	1	3	3
7d	2	1	1	2
Mean 24/48/72h	1.33	1	2.67	3.33
Revers (72h – 7d)	N	N	Y↓	Y↓

	Rabbit 2			
			Conjunctiva	
	C	I	E	O
24h	2	1	2	4
48h	2	1	3	3
72h	3	1	3	3
7d	3	1	2	3
Mean 24/48/72h	2.33	1	2.67	3.33
Revers (72h – 7d)	N	N	Y↓	N

	Rabbit 3			
			Conjunctiva	
	C	I	E	O
24h	2	1	3	4
48h	2	1	3	4
72h	2	1	3	4
7d	2	1	2	2
Mean 24/48/72h	2	1	3	4
Revers (72h – 7d)	N	N	Y↓	Y↓

	Rabbit 4			
			Conjunctiva	
	C	I	E	O
24h	2	1	2	4
48h	3	1	2	4
72h	3	1	3	4
7d	2	1	2	2
Mean 24/48/72h	2.67	1	2.33	4
Revers (72h – 7d)	Y↓	N	Y↓	Y↓

	Rabbit 5			
			Conjunctiva	
	C	I	E	O
24h	3	1	2	4
48h	3	1	2	4
72h	3	1	3	4
7d	2	1	2	2
Mean 24/48/72h	3	1	2.33	4
Revers (72h – 7d)	Y↓	N	Y↓	Y↓

	Rabbit 6			
			Conjunctiva	
	C	I	E	O
24h	2	1	2	4
48h	1	1	2	3
72h	2	1	2	2
7d	2	1	2	2
Mean 24/48/72h	1.67	1	2	3
Revers (72h – 7d)	N	N	N	N

N = same score; Y↓ = decreased score

Comparison with the criteria

The calculated mean scores following grading at 24, 48 and 72 hours after instillation in study B.6.2.5-01 fulfil the criteria for classification as eye irritant for the three rabbits: corneal opacity (≥ 1) and conjunctival oedema (≥ 2), but are not high enough to fulfil the score criteria for classification as serious eye damage (Eye Dam. 1). However, CLP criteria for classification of substances within hazard class Category 1 (serious eye damage), includes persistent lesions (those that are not fully reversible within an observation period of normally 21 days). In this case, the study was finalised after 8 days. RAC notes that at this point, the scores for corneal opacity and iritis were not lower than the previous observation time, the corneal opacity reached a grade 3 in one animal, and for two animals, the severity of iritis increased (from grade 1 after 72h, to grade 2 after 8 days). Moreover, the grade of iritis remaining after 8 days is considered severe in the three rabbits, since this score (2) exceeds the value established as CLP criteria (> 1.5 mean value 24/48/72h) for classification of substances as Category 1.

Similar results are observed in the study B.6.2.5-03, where reversibility of the lesions was not proven since the study was finalized after 7 days, when corneal and iris lesions (in 4 and 5 rabbits, respectively) presented the same grade of severity than 72h after the instillation. The calculated mean scores following grading at 24, 48 and 72 hours after instillation of the test material, for the six rabbits fulfil the criteria for classification as eye irritant: corneal opacity (≥ 1), iritis (≥ 1), and conjunctival redness (≥ 2) and oedema (≥ 2). However, the study was finalised after 7 days and the reversibility of the lesions could not be proved. At the end of the study, scores for corneal opacity in 4 rabbits and iritis scores of the 6 rabbits were the same as those registered at 72 hours post-instillation (grades 2 and 3 for cornea, and grade 1 for iritis), showing serious eye irritation effects with no reversibility after 7 days.

In summary, there are two studies showing severe ocular lesions, non-reversible after up to 8 days; which strictly speaking is not enough for demonstrating irreversibility after 21 days of observation but strongly suggest that the substance is able to cause serious eye damage. Moreover, it should be noted that, according to the CLP guidance, if a substance is classified as Skin Corr. 1, as this is the case of OPP, then serious damage to eyes is implicit, as reflected in the hazard statement for skin corrosion (H314: Causes severe skin burns and eye damage). Thus, the classification of OPP for serious eye damage is warranted and **RAC supports the DS's proposal for classification of OPP as Eye Dam. 1; H318, causes serious eye damage**.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

DS proposed no classification for respiratory sensitisation due to lack of data.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

No classification due to lack of data is supported.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

DS proposed no classification of OPP for skin sensitisation based on the lack of positive results in animals and the questionable quality and reliability of human data.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Animal data

The table below summarises the results of the skin sensitisation studies with animals. The outcome of the three animal studies suggests no evidence of skin sensitization, although all the studies have deviations that do not allow to grant full reliability to none of them.

Table: *Summary of the animal studies on skin sensitisation with OPP*

<table>
<tr><th>Study</th><th>Dose level</th><th>Results</th><th>Reference</th></tr>
<tr><td>Skin sensitization in guinea pig

US EPA 81-6

Buehler method

Comparable to OECD TG 406

GLP: Yes

Male Hartley Guinea Pigs

Test group: 10

Positive control group: 10</td><td>0.4 g (100% solid) for induction and challenge phases

Positive control: DER 331 epoxy resin: 10% for induction and 7.5 for challenge</td><td><table><tr><td></td><td>Test group</td><td>Positive control</td></tr><tr><td>24h</td><td>0/10</td><td>8/10</td></tr><tr><td>48h</td><td>0/9*</td><td>9/10</td></tr></table>* One animal had to be euthanized because of a non-treatment-related injury.

Deviations: only 10 animals treated; no negative control group.</td><td>B.6.2.6-01, 1991</td></tr>
<tr><td>Skin sensitization in guinea pig

OECD TG 406 (1987)

Buehler method

GLP: Yes

Deviations: only 10 animals in the treated group; only 5 animals in the control group</td><td>Induction: 0.4 g moistened with 0.2 mL water

Challenge: 75% aqueous suspension

Positive: DER 331 epoxi resin: 10% induction and challenge</td><td><table><tr><td>Induction</td><td colspan="2">None</td><td>100% OPP</td><td>10% DER 331</td></tr><tr><td>Challenge</td><td>7.5% OPP</td><td>10% DER 331</td><td>7.5% OPP</td><td>10% DER 331</td></tr><tr><td>Time</td><td colspan="4"></td></tr><tr><td>24h</td><td>0/5</td><td>1*/5</td><td>0/10</td><td>10**/10</td></tr><tr><td>48h</td><td>0/5</td><td>0/5</td><td>0/10</td><td>9***/10</td></tr></table>*Erythema grade 1 (slight): may have been due to a scratch

**Erythema grade 1 (slight) in 5 animals and grade 2 (moderate) in 6 animals

***Erythema grade 1 (slight) in 7 animals and grade 2 (moderate) in 2 animals</td><td>B.6.2.6-02, 1994b</td></tr>
</table>

<table>
<tr><th>Study</th><th>Dose level</th><th>Results</th><th>Reference</th></tr>
<tr><td>Skin sensitization in guinea pig

Similar to OECD TG 406 (GPMT)

GLP: No

Guinea Pig

Test group: 20

Control group: 20</td><td>Intradermal induction: 0.5 or 5%

Topical induction: 25%

Challenge: 5%</td><td><table><tr><th>Test compound</th><th>Induction [%] (intradermal + topical)</th><th>Frequency of positive challenge on day 21</th></tr><tr><td>OPP</td><td>0.5 + 25</td><td>0/20</td></tr><tr><td></td><td>5 + 25</td><td>0/20</td></tr></table>
Deficiencies: test substance not characterised; no individual results reported. Grade 1 reactions were omitted in the analysis of the test results</td><td>Andersen and Hamann, 1984

B.6.2.6-03</td></tr>
</table>

In addition to the studies summarised in the table above, RAC notes that the Draft Renewal Assessment Report also contains a local lymph node assay (LLNA) in mice with AGF/1-04, which is a representative biocidal formulation containing 10% OPP (KCP 7.1.6/01, 2005). The study was performed in compliance with GLP and OECD TG 429 with the following deviations: 1) the measurement of cell proliferation was achieved by cell counting instead of determination of ^{3}H-thymidine incorporation; 2) the animals were sacrificed on the day after the last treatment (day 4) instead of day 6; 3) neither data on the followed procedure nor the results of the most recent positive control group are included in the study report. In this study, AGF/1-04 did not show an increased lymph node cell count at test concentrations of up to 50%.

Human data

The table below summarises human data on skin sensitisation. Among negative results obtained in several studies, few positive skin sensitisation cases were also reported. This positive results on skin sensitization in humans cannot be overlooked, but important information is lacking, like the followed procedure (e.g. purity of the test substance or vehicle used on the administered patches) or if there was a clear discrimination between irritant and sensitization skin reactions (this point is considered important since OPP is corrosive to the skin).

The study B.6.2.6-04 shows no evidence of skin sensitization after the application of OPP to 200 unselected human subjects (both sexes). This publication can be considered as supportive information because it cannot be assumed that it was a properly conducted Human Repeat Insult Patch Test. According to the available data on occupational medical surveillance (B.6.9.1) that used the data of 65 employees (examined every 3 years between 2004 and 2018), no indications of skin sensitisation for OPP among employees were observed.

Data collected on humans (B.6.9.2) include the description of three cases of skin sensitization in patients whose contact with OPP was suspected to be at work (coolant, or a germicidal agent) and one unusual case of contact urticarial related with the OPP found in one of the components of a plaster.

Epidemiological studies (B.6.9.4) altogether showed a low sensitizing potential of OPP, with positive reactions in 0.29% to 0.72% of the study subjects. Most of the data obtained comes from metalworkers or patients of dermatological clinics who, in most cases, already presented skin problems (dermatitis, assumed occupational dermatosis or suspected allergic contact dermatitis). Besides, there is no information on the specifications of the substance applied. Although the patch test was performed in all of them with OPP at a concentration of 1% in petrolatum, different exposure periods were used (i.e. 24 or 48h depending on the study/test facility), which makes it more difficult to compare possible results. Only one study (Brasch *et al*., 1993) described different reading time points (after removal of the patch and on the following two days).

***Table**: Summary of human data on skin sensitisation with OPP*

Type of data/report	Test substance	Relevant information and results	Reference
Skin sensitization in humans 200 unselected human subjects (100/sex)	5% in sesame oil 1st application: 5 days in contact 2nd application (3 weeks later): 48h in contact.	Readings: after removal of both patches, and days 3 and 8 after removal of the 2nd patch. OPP did not cause primary irritation when tested as a 5% solution in sesame oil nor did it cause any sensitisation.	Hodge *et al*., 1952 B.6.2.6-04
Occupational medical surveillance on manufacturing plant personnel	OPP	Report not provided Occupational medical surveillance of workers potentially exposed to OPP is performed in 3-year intervals on a routine basis. 65 employees, examined every 3 years, between 2004 and 2018: there were no indications for airway or skin sensitisation towards OPP among employees.	Leng, 2019 B.6.9.1
Allergic contact dermatitis due to OPP	Patch testing with 0.5% or 1% OPP	Description of 2 cases of patients with allergic contact dermatitis due to OPP Extensive and severe dermatitis in both cases Patch testing with 0.5% or 1% OPP, respectively, gave positive result in both individuals.	Adamds, 1981 B.6.9.2
Contact urticarial to OPP	OPP	Single case. Unusual presentation of contact urticarial in the form of an immediate reaction to a component of plaster cast material. Several components of the plaster were tested separately by topical application of a 1% solution, resulting in a localised reaction within ten minutes at the site where the preservative OPP had been placed. Similar challenge in 20 control subjects produced no reactions.	Tuer *et al*., 1986 B.6.9.2
Contact sensitivity to OPP in a coolant	OPP	Case report of one individual with dermatitis related to his work. Machinist worked in contact with coolant liquid (containing OPP) and sometimes a cleaner (containing sodium OPP salt) was added to the coolant. Testing with coolant revealed sensitivity to OPP. OPP (1% in petrolatum) and the cleaner caused redness, oedema and vesicles.	Van Hecke, 1986 B.6.9.2
Epidemiological study	OPP Exposition for	Patch test reactions to several industrial biocides (OPP was one of the tested ones).	Geier *et al*., 1996

Type of data/report	Test substance	Relevant information and results	Reference
Contact allergies caused by industrial biocides	48h (in 732 patients) or 24h (in 400) Skin reaction was scored 72h after application of the patch.	1132 patients from dermatological clinics. The largest group (28.5%) were employed in metal industry. In 497 cases (43.9%), an occupational dermatosis was assumed. 5 individuals (0.40%) showed positive reactions 1 individual showed irritation 1 individual showed ambiguous result	B.6.9.4
Retrospective study based on data collected by the IVDK	OPP 2043 patients tested with 1% OPP in petrolatum Exposition for 24 or 48 hours Readings at removal of the patch and on the following two days	More than 40000 patch test reactions against a set of 24 medical preservatives in 2059 patients, recorded between 1989 and 1991 6 (0.29%) showed weak to medium positive reactions to OPP In 8 cases (0.39%) the reaction was equivocal 1 individual (0.05%) displayed irritant reaction	Brasch *et al*., 1993 B.6.9.4
Dermatoses in metal workers (II) Allergic contact dermatitis	OPP Patch tests were also performed with OPP (1% in petrolatum) 48h exposure (occlusion) Scorings: 72h after patches application	Epidemiological study (in 10 metalworking factories): the prevalence of contact sensitisation was investigated in 286 metalworkers exposed to metalworking fluid. Several workers presented skin lesions at the time of the investigations. 8 workers of 286 (2.7%) showed contact allergy None of these cases were related to OPP	De Boer *et al*., 1989 B.6.9.4
Contact Allergy in Metal Workers – 1-year analysis based on data collected by IVDK	OPP 1% in petrolatum Exposition for 48h (occlusion) Scoring at 72h after the patches were applied	Epidemiological study to investigate the prevalence of contact sensitisation in 424 metalworkers exposed to metalworking fluid. 2 test series: - additives industrial fluids (included OPP) - common components of metalworking fluid 277 patients received an application of 1% OPP in petrolatum 2 individuals showed a positive reaction (0.72%)	Uter *et al*., 1993 B.6.9.4

Type of data/report	Test substance	Relevant information and results	Reference
Patch testing with preservatives, antimicrobials and industrial biocides	OPP 1% in petrolatum Exposure for 24 or 48h Readings 72h after application	The role of different preservatives (OPP included) in a large number of patients with suspected allergic contact dermatitis was examined 11485 patients tested with a preservative series containing OPP at a concentration of 1% in petrolatum 33 subjects (0.3%) were positive 59 subjects (0.5%) showed an irritative or questionable result	Geier *et al.*, 1998 B.6.9.4

Comparison with the criteria

The CLP guidance establishes that the relatively high or low frequency of occurrence of skin sensitisation can be determined attending the following considerations:

Human diagnostic patch test data	High frequency	Low/moderate frequency
General population studies	≥ 0.2%	< 0.2%
Dermatitis patients (unselected, consecutive)	≥ 1.0%	< 1.0%
Selected dermatitis patients (aimed testing, usually special test series)	≥ 2.0%	< 2.0%
Workplace studies:		
1: all or randomly selected workers	≥ 0.4%	< 0.4%
2: selected workers with known exposure or dermatitis	≥ 1.0%	< 1.0%
Number of published cases	≥ 100 cases	< 100 cases

The first study summarised in the table above (Hodge *et al.*, 1952) is a study among the general population where no positive cases were reported among 200 unselected human patients. Similarly, no positive cases were determined in the study at a workplace with selected workers with known exposure or dermatitis among 65 occupationally exposed individuals (Leng, 2019).

The table above includes 3 different studies (Adamds, 1981; Tuer *et al.*, 1986, and Van Hecke, 1986) with 4 case reports. No information about frequency can be obtained from these cases, but they would score within the last entry of the above table (number of published cases).

All the remaining four studies summarised in the table, depending on which aspects the attention is focused, could be fitted as selected dermatitis patients, workplace studies with randomly selected workers or work place studies with selected workers with known exposure or dermatitis. However, in no case any study can be fitted one specific category because all of them seems to be performed under occupational premises but, for example, in Geier *et al.* (1996) the patients seem to be taken from dermatological clinics and moreover patients with occupational dermatosis seem to be pooled with those without this disease. Overall, in a conservative approach RAC would assess the frequency as workplace studies with all or randomly selected workers.

The estimated frequency of occurrence of skin sensitisation in Geier *et al*. (1996) should be considered as high since 0.4% of individuals showed positive reactions. Similar result (high frequency) is obtained in the De Boer *et al*. (1989) and Uter *et al*. (1993) studies, where 2.7% and 0.72% of tested workers showed positive reactions. On the contrary, in Brasch *et al*. (1993) and Geier *et al.* (1998) frequencies of skin sensitisation were low because the occurrences were 0.29% and 0.3%; respectively.

RAC notes that the number of patients in all these studies is very different and therefore to improve the comparisons among all of them the results of all these studies were pooled and the results are shown below.

Total individuals	Positive cases	Study
200	0	Hodge *et al.,* 1952
65	0	Leng, 2019
1132	5	Geier *et al.,* 1996
2059	6	Brasch *et al.,* 1993
286	8	De Boer *et al.,* 1989
277	2	Uter *et al.,* 1993
11485	33	Geier *et al.,* 1998
15504	54	**TOTAL (0.3%)**

Overall, the frequency of occurrence of skin sensitisation is 0.3% (54 cases among 15504 exposed people); which amounts to a low frequency of occurrence. The last criterion for assessing the occurrence is the number of published cases, that is of 58 (54 showed above plus 4 in case reported); which is lower than 100 and scores as low frequency too. In conclusion, the weight of evidence suggests that potential of OPP for inducing skin sensitisation would be low.

The CLP guidance also provides criteria for determining whether the cases of skin sensitisation occur at relatively low or relatively high exposure. RAC notes that such criteria cannot be applied with the available information because only the number of exposures (irrespective of the concentration of the sensitiser) are known; but not the concentration/dose or the possible repeated exposure.

The CLP guidance establishes that substances showing a high frequency of occurrence in humans or a high potency in animals shall be considered for classification within category 1A. There are no positive studies in animals and the frequency of occurrence of skin sensitisation in humans is, with the available information, lower than 100 cases and with a frequency of approximately 0.3%; which are records considered for skin sensitisers of low frequency. Therefore, the conditions for classification of OPP as skin sensitiser category 1A have not been met.

However, substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans and shall be classified as skin sensitiser category 1B. The frequency of skin sensitisation occurrence in humans (0.3%) suggests a low frequency and therefore category 1B is warranted. In conclusion, **RAC proposes the classification of OPP as Skin Sens. 1B; H317, may cause an allergic skin reaction**, with GCL of 1% (w/v).

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS identified urinary bladder as target organ for OPP. However, proposed no classification because the effects reported on the available studies were observed at concentrations above the threshold values considered for triggering classification.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The table below summarises all available repeated dose toxicity studies in animals with OPP. A number of them covering oral dosage regimen from 13-days to 1-year and using rats, dogs and rabbits (B.6.3.1-01, B.6.3.1-02, B.6.3.1-03, B.6.3.1-04, B.6.3.2-01, B.6.3.2-04) were unable to identify a target organ and only minor unspecific signs of toxicity were reported. The table also summarises the results of two studies covering dermal exposure (B.6.3.3-01 and B.6.3.3-02). In these two studies, skin irritation hyperkeratosis, acanthosis, and ulcerative skin lesions were reported. RAC notes that OPP is corrosive and therefore these effects can be considered local rather than systemic and consequently cannot be considered for setting a classification as STOT RE.

Table: *Summary for repeated dose toxicity studies in animals with OPP. Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr).*

<table>
<tr><th>Method</th><th>Results</th><th>Reference</th></tr>
<tr><td>1-month dietary

No guideline

Rats of unspecified strain

5 females/dose

OPP (98% purity)

Dietary: 0, 2000, 3000, 4000, 5000 and 10000 mg/kg bw/day</td><td><u>Mortality:</u> all deaths occurred within 2 weeks

<table><tr><th>Dose (mg/kg bw/day)</th><th>Mortality</th></tr><tr><td>2000</td><td>0/5</td></tr><tr><td>3000</td><td>4/5</td></tr><tr><td>4000</td><td>5/5</td></tr><tr><td>5000</td><td>5/5</td></tr><tr><td>10000</td><td>5/5</td></tr></table>
<u>Clinical signs:</u> slight growth retardation was seen in the 2000 mg/kg bw/day group, all of the other dose groups lost weight rapidly

LOAEL = 2% (2000 mg/kg bw/day)

NOAEL < 2% (2000 mg/kg bw/day)

Target organs/tissues were not identified

Critical effect at the LOAEL: growth retardation</td><td>Hodge et al., 1952

B.6.3.1-01</td></tr>
<tr><td>32-day oral

No guideline

15 White male rats/dose

OPP

Oral gavage: 0, 2, 20, 200 mg/kg bw/day, for 32-days</td><td>There were no reported adverse effects attributable to OPP administration

LOAEL > 200 mg/kg bw/day

NOAEL = 200 mg/kg bw/day

Target organs/tissues were not identified</td><td>Macintosh, 1945

B.6.3.1-02</td></tr>
<tr><td>13-day oral

EPA FIFRA 83-3(b) but checked for compliance with OECD TG 407

Deviations: only females and only 2 animals per dose; haematology, clinical chemistry, and</td><td><u>Mortality</u>
• In the high dose group, 1 rabbit died on test day 8 and 1 rabbit was sacrificed moribund on test day 10

<u>Clinical signs</u>
• Decreased amount of faeces was observed in all the treated animals with ≥ 500 mg/kg bw/day)
• One 500 mg/kg bw/day animal, showed laboured respiration, moist rales and perineal soiling due to aspirated test material

<u>1000 mg/kg bw/day</u></td><td>B.6.3.1-03, 1991a</td></tr>
</table>

Method	Results	Reference
histopathology not conducted 2 NZW female rabbits /dose OPP (99.77% purity) Oral gavage: 0, 100, 500 or 1000 mg/kg bw/day	• ↓ final body weight (25%) • Decrease in food consumption (2/2, n.s.; no numerical data available) <u>500 mg/kg bw/day</u> • ↓ final body weight (6.3%, n.s.) • ↑ absolute/relative, kidney weight (11.5%, n.s./19.2%, n.s.) • ↓ absolute/relative, liver weight (20%, n.s., ndr/15%, n.s., ndr) • Decrease in food consumption (2/2, n.s.; no numerical data available) <u>100 mg/kg bw/day</u> • ↓ absolute/relative, liver weight (26%, n.s., ndr/24%, n.s. ndr) LOAEL = 500 mg/kg bw/day NOAEL = 100 mg/kg bw/day **Target organs/tissues were not identified** Critical effect at the LOAEL: ↓ in body weight, body weight gain and amount of fat and ↑ absolute and relative kidneys weights.	
4-week oral No guideline 2 Beagle dogs /dose/sex OPP (99.77% purity) Oral gavage: 0, 100, 200, 300 (400 mg up to day 5, lowered to 300 due to emesis) mg/kg bw/day 5 days a week for four weeks	<u>General observations</u> • Dose-related emesis in all dogs (♂ and ♀) treated with ≥ 200 mg/kg bw/day • No deaths occurred throughout the study at any dose tested <u>Bodyweight</u> • No differences in body weight were found compared with controls <u>Haematology</u> *300 mg/kg bw/day* • ↓ RBC (25%, n.s.) in ♂ • ↓ HGB (20%, n.s.; ndr.) in ♂ • ↓ HCT (22%, n.s.) in ♂ • ↓ Platelet in ♂ (34%, n.s.; ndr.) and ♀ (7%, n.s.; ndr.) *200 mg/kg bw/day* • ↓ RBC (11%, n.s.) in ♂ • ↓ HCT (9%, n.s.) in ♂ *100 mg/kg bw/day* • ↓ RBC (6%, n.s.) in ♂ • ↓ HCT (9%, n.s.) in ♂ LOAEL = 200 mg/kg bw/day NOAEL = 100 mg/kg bw/day **Target organs/tissues were not identified** Critical effect at the LOAEL: repeated emesis	B.6.3.1-04, 1990
3-month dietary No guideline	<u>2000 mg/kg bw/day</u> • Slight growth retardation (no detailed data provided in the study)	Hodge *et al.*, 1952 B.6.3.2-01

Method	Results	Reference
Rats of unspecified strain. 12 rats/sex/dose OPP (≥ 98% purity) Dietary: 0, 100, 300, 1000 and 2000 mg/kg bw/day	• ↑ liver, kidney and spleen weight (n.s.). in some rats (no numerical data available) <u>1000 mg/kg bw/day</u> • ↑ liver, kidney and spleen weight in some rats (no numerical data available) LOAEL = 2000 mg/kg bw/day NOAEL = 1000 mg/kg bw/day **Target organs/tissues were not identified** Critical effect at the LOAEL: ↓ in body weight	
6-month dietary No guideline Rats of unspecified strain. 12 rats/sex/ dose OPP (≥98% purity) Oral gavage: 0, 50, 100, 200, 500 mg/kg bw/day 5 days/week	<u>500 mg/kg bw/day</u> • ↑ liver and kidney weight (no numerical data available) LOAEL = 500 mg/kg bw/day NOAEL = 200 mg/kg bw/day **Target organs/tissues were not identified** Critical effect at the LOAEL: ↑ liver and kidney weight	Hodge *et al.*, 1952 B.6.3.2-01
13-week dietary No guideline but it is similar to OECD TG 408. Deviations: no neurobehavioralal examinations; no detailed reporting 10 F344/DuCrj rats/sex/dose OPP (98% purity) Dietary: ♂/♀ : 0/0, 182/202, 391/411, 761/ 803, 1669/1650, and 2798/3014 mg/kg bw/day	<u>Mortality</u> • In the high dose group, 2♂ died on day 4 and 1♀ died on day 8. <u>♂/♀ (2798/3014 mg/kg bw/day)</u> *Bodyweight and food/water consumption* • ↓ body weight in ♂/♀ [throughout the study (from 27 to 44%/from 20 to 30%)] • ↓ in terminal body weight in ♂/♀ (11%,/22%). • ↓ body weight gain in ♂/♀ (first week 35/31% for ♂/♀)] • ↓ food consumption (absolute weight) in ♂/♀ [week 0 (83%/80%), week 3 (22%/ 23%) , week 6 (27%/18%), week 9 (29%/-) and week 12 (27%/-)] • ↓ water consumption (absolute weight) in ♂/♀ [week 0 (53%/54%) and week 2 (13%/-)] and ↑ water consumption in ♀ [week 12 (32%)] *Urinalysis* • Occult blood in ♂ [week 9 (1/6 vs 0/10 in controls, n.s.) and week 13 (1/8 vs 0/8 in controls, n.s.)] • ↓ pH in ♂/♀ [week 9 and week 13] *Haematology* • ↓ RBC in ♂ (5%) • ↓ Hg in ♂/♀ (6.8%/6%) • ↓ MCV in ♀ (2%) • ↓ MCH in ♂/♀ (2%, ndr/7%) • ↓ MCHC in ♂ (5%)	Iguchi *et al.*, 1984 B.6.3.2-02

Method	Results	Reference
	Organ weight • Liver: ↑absolute weight in ♀ (17%, ndr) and ↑ relative weight in ♂/♀ (20%/33%) • Thymus: ↓absolute weight in ♂/♀ (24%, ndr/9%, ndr) • Spleen: ↓ absolute weight in ♂/♀ (14%/9%, ndr) and ↑ relative weight in ♂ (9%) • Kidney: ↑ relative weight in ♂/♀ (25%/15%) • Adrenals: ↓ absolute weight in ♂ (15%, ndr) and ↑ relative weight in ♂/♀(13%, ndr/10%, ndr) • Bladder: ↑ relative weight in ♂ (60%) <u>Histopathology</u> • Inflammation of the kidneys in ♂/♀. • Abnormal growth in the bladder mucosa in ♂ <u>♂/♀ (1669/1650 mg/kg bw/day)</u> *Bodyweight and food/water consumption* • ↓ body weight in ♀ [from week 1 to 8 (7 to 10%)] • ↓ food consumption in ♂ [week 0 (8%, ndr)] • ↓ water consumption in ♂/♀ [week 0 (13%/13%)] *Urinalysis* • Occult blood in ♂ [week 13 (1/8 vs 0/8 in controls, n.s.)] *Haematology* • ↓ Hg in ♀ (4%) • ↓ MCH in ♀ (3%) *Organ weight* • Liver: ↑ relative weight in ♂/♀ (11%/13%) • Kidney: ↑ relative weight in ♂ (6%) • Bladder: ↑ absolute weight in ♂ (40%, ndr) and ↑ relative weight in ♂ (49%) <u>Histopathology</u> • Abnormal growth in the bladder mucosa in ♂ <u>♂/♀ (761/ 803 mg/kg bw/day)</u> • Liver: ↑ relative weight in ♂ (7%) • Thymus: ↓ relative weight in ♂ (13%, ndr) and ↑ relative weight in ♀ (10%, ndr) • Kidney: ↑ relative weight in ♂ (4%) <u>♂/♀ (391/411 mg/kg bw/day)</u> • Liver: ↑ absolute/relative weight in ♂ (19%, ndr/7%) LOAEL = 1669 mg/kg bw/day NOAEL = 761 mg/kg bw/day **Target tissue/organ: kidneys urinary bladder** Critical effect at the LOAEL: ↑ relative bladder weights (♂) with onset of abnormal urothelial growth.	

Method	Results	Reference
One-year oral No guideline but it is similar to OECD TG 409 Deviations: Only 4 animals per group OPP (99.77% purity) Oral gavage: 0, 30, 100, 300 mg/kg bw/day 4 Beagle dogs/sex/dose	Mortality • Two high-dose ♂ died after test days 137 and 138 due to the inadvertent deposition of dosing solution into the lungs General observations • Dose-related emesis in all dogs (♂ and ♀) treated with ≥ 100 mg/kg bw/day during the entire dosing period 300 mg/kg bw/day *Bodyweight* • ↓ Terminal body weight in ♀ (8%, n.s). *Clinical chemistry* • ↓ Creatinine phosphokinase (CPK) in ♂ (46%). *Gross pathology* • The two dogs that died had dark regions in the pulmonary parenchyma, which is consistent with administration of test material into the lungs, resulting in anoxia/shock LOAEL = 300 mg/kg bw/day NOAEL = 100 mg/kg bw/day **Target organs/tissues were not identified** Critical effect at the LOAEL: ↑ emesis	B.6.3.2-03, 1990
One-year Oral No guideline but it is similar to OECD TG 409. Deviations: only 1 or 2 animals per dose level, test substance not characterised, no clinical chemistry 1 to 2 dogs (unspecified strain)/sex/dose OPP (≥ 98% purity) 0, 20, 200, 500 mg/kg bw/day	Mortality: The single male treated with 500 mg/kg bw/day was terminated after 6 months because of serious illness, which was found to be not treatment-related 500 mg/kg bw/day *Organ weight* • ↑ kidney weight ♂ (no numerical data) LOAEL = 500 mg/kg bw/day NOAEL = 200 mg/kg bw/day **Target organs/tissues were not identified** Critical effect at the LOAEL: ↑ kidney weight	Hodge *et al.*, 1952 B.6.3.2-04
21-day Dermal EPA FIFRA 82-2, MAFF OECD TG 410	1000 mg/kg bw/day *Gross pathology* • ↑ Incidence of local skin irritation in ♂ (2/5 vs 0/5 in control) and ♀ (5/5 vs 0/5 in control) *Histopathology* • ↑ Incidence of hyperkeratosis and acanthosis in ♂ (3/5 vs 0/5 in control) and ♀ (4/5 vs 0/5 in control)	B.6.3.3-01, 1993

Method	Results	Reference
5 Fischer 344 rats/sex/dose 5 days/week for 21-days 0, 100, 500 and 1000 mg/kg bw/day	500 mg/kg bw/day *Gross pathology* • ↑ Incidence of local skin irritation in ♀ (1/5 vs 0/5 in control) *Histopathology* • ↑ Incidence of hyperkeratosis and acanthosis in ♂ (1/5 vs 0/5 in control) and ♀ (4/5 vs 0/5 in control) Local/dermal LOAEL = 500 mg/kg bw/day Local/dermal NOAEL = 100 mg/kg bw/day Critical effect at the dermal LOAEL: local irritation at the application site in ♀ and associated histopathology in ♂ and ♀ at 500 mg/kg bw/day Systemic LOAEL > 1000 mg/kg bw/day Systemic NOAEL = 1000 mg/kg bw/day Critical effect at the systemic LOAEL: no systemic effects in any group **Target organs/tissues were not identified**	
4-week Dermal No guideline but it is similar to OECD TG 409 Deviations: equivalence between amounts of test substance applied and dose level in mg/kg bw/day was not reported, food consumption not measured, haematology and clinical chemistry were not performed, organs were not weighed. 10 Swiss Webster CFW mice/sex/dose OPP (> 99% purity) 0, 5.95, 11.4, 20.8, 35.7, 55.5 mg/0.1 mL acetone 3 days/week for 4-weeks	Ulcerative lesions at the site of application were observed in all mice that received ≤ 20.8 mg OPP; in 6/10 males and 9/10 females that received 11.4 mg; in 2/10 males and 7/10 females that received 5.95 mg, and in 1/10 male and 1/10 female of control group LOAEL = 5.95 mg (equivalent to 200 /240 mg/kg bw/day, ♂/♀) NOAEL < 5.95 mg or 200 /240 mg/kg bw/day ♂/♀ **Target organs/tissues were not identified** Critical effect at the LOAEL: occurrence of local ulcerative skin lesions (♂, ♀ but females are seemingly more sensible); no systemic effects	National Toxicology Program (NTP), 198 B.6.3.3-02

Method	Results	Reference
Combined chronic toxicity/carcinogenicity 20 Fischer 344 rats/sex/dose in the 1 year-group 50 Fischer 344 rats /sex/dose in the 2 year-group OPP (purity 99.7-100%) 39/49, 200/248 and 402/647 mg/kg bw/day for ♂/♀ for 2-years	<u>402/647 mg/kg bw/day (♂/♀)</u> *Gross pathology* ↑ Incidence of urinary bladder masses in ♂ (74% vs 0% in controls) ↑ Incidence of pitted zones in kidneys in ♀ (14% vs 0% in controls) *Non-neoplastic changes: urinary bladder* • ↑ Incidence of nodular/papillary hyperplasia in ♂ at 12 months (20/20 vs 0/20 in controls) and 24 months (43/50 vs 1/50 in controls) • ↑ Incidence of simple hyperplasia in ♂ at 12 months (20/20 vs 0/20 in controls) and in ♂/♀ at 24 months (42/50 vs 2/50 in control ♂ /6/50 vs 0/50 in control ♀, respectively) • ↑ Incidence of calculus in ♂ at 12 months (16/20 vs 8/20 in controls), and at 24 moths (21/50 vs 3/50 in controls) • ↑ Incidence of congestion in ♂at 24 moths (16/50 vs 1/50 in controls) • ↑ Incidence of haemorrhage in ♂ at 24 moths (9/50 vs 0/50 in controls) • ↑ Incidence of mineralisation in ♂ at 24 moths (18/50 vs 3/50 in controls) • ↑ Incidence of necrosis in ♂ at 24 moths (20/50 vs 3/50 in controls) • ↑ Cyst in ♀ at 12 months (5/20 vs 0/20 in controls) *Non-neoplastic changes: Kidney* • ↑ Incidence calculus in ♀ at 24 months (21/50 vs 16/50, n.s.; ndr) • ↑ Incidence cysts in ♂/♀ at 24 months (17/50 vs 4/50 in control ♂; ncdr; 37/50 vs 14/50 in control ♀, ndr, respectively) • ↑ Incidence hyperplasia in ♀ at 24 months (30/50 vs 3/50 in controls) • ↑ Incidence infarct in ♀ at 24 months (29/50 vs 3/50 in controls) • ↑ Incidence acute inflammation in ♀ at 24 months (11/50 vs 2/50 in controls) • ↑ Incidence papilla mineralization in ♀ at 24 months (12/50 vs 0/50 in controls) <u>200/248 mg/kg bw/day (♂/♀)</u> *Gross pathology* • ↑ Incidence of urinary bladder masses in ♂ (4% vs 0% in controls; n.s.) *Non-neoplastic changes: urinary bladder* • ↑ Incidence of simple hyperplasia in ♂ at 24 months (6/50 vs 2/50 in control; n.s.) Systemic LOAEL = 200 mg/kg bw/day Systemic NOAEL = 39 mg/kg bw/day Critical effect at the LOAEL: structural alterations in the urinary bladder (♂) **<u>Target tissue/organ: urinary bladder</u>**	B.6.5-02, 1996

Method	Results	Reference
Dietary in mouse 60 B6C3F1 mice /sex/dose OPP (purity 99.88%) 0, 250, 500, 1000 mg/kg bw/day for 2-years	<u>1000 mg/kg bw/day</u> *Non-neoplastic changes: Liver* • ↑ Accentuated lobular pattern (slight) in ♂ (22%; 11/50 animals vs 6%; 3/50 in controls), and ♀ (38%; 19/50 animals vs 4%; 2/48 in controls) • ↑ Accentuated lobular pattern (moderate) in ♂ (52%; 26/50 animals vs 2%; 1/50 in controls), and ♀ (28%; 14/50 animals vs 4%; 2/48 in controls) • ↑ Accentuated lobular pattern (any severity) in ♂ (74%; 37/50 animals vs 24%; 12/50 in controls), and ♀ (74%; 37/50 animals vs 15%; 7/48 in controls) • ↑ Focus of altered cells-eosinophilic, hepatocellular, multifocal in ♂ (18%; 9/50 animals vs 2%; 1/50 in controls) • ↑ Focus of altered cells-eosinophilic, hepatocellular, focal or multifocal in ♂ (32%; 16/50 animals vs 6%; 3/50 in controls) *Non-neoplastic changes: Kidney* • Degeneration/regeneration tubule (very slight) in ♂(76%; 38/50 animals vs 34%; 17/50 in controls) • ↑ Vacuolation decreased tubule (moderate) in ♂ (42%; 21/50 animals vs 2%; 1/50 in controls) • ↑ Vacuolation decreased tubule (severe) in ♂ (58%; 29/50 animals vs 12%; 6/50 in controls) • ↑ Vacuolation decreased tubule (any severity) in ♂ (100%; 50/50 animals vs 30%; 15/50 in controls) <u>500 mg/kg bw/day</u> *Non-neoplastic changes: liver* • ↑ Accentuated lobular pattern (slight) in ♂ (40%; 20/50 animals vs 6%; 3/50 in controls), and ♀ (20%; 10/50 animals vs 4%; 2/48 in controls) • ↑ Accentuated lobular pattern (moderate) in ♂ (22%; 11/50 animals vs 2%; 1/50 in controls) • ↑ Accentuated lobular pattern (any severity) in ♂ (70%; 35/50 animals vs 24%; 12/50 in controls), and ♀ (52%; 26/50 animals vs 15%; 7/48 in controls) • ↑ Focus of altered cells-eosinophilic, hepatocellular, focal or multifocal in ♂ (24%; 12/50 animals vs 6%; 3/50 in controls) *Non-neoplastic changes: kidney* • ↑ Degeneration/regeneration tubule (very slight) in ♂(68%; 34/50 animals vs 34%; 17/50 in controls) • ↑ Vacuolation decreased tubule (moderate) in ♂ (62%; 31/50 animals vs 2%; 1/50 in controls) • ↑ Vacuolation decreased tubule (severe) in ♂ (28%; 14/50 animals vs 12%; 6/50 in controls) • ↑ Vacuolation decreased tubule (any severity) in ♂(100%; 50/50 animals vs 30%; 15/50 in controls) <u>250 mg/kg bw/day</u> *Non-neoplastic changes: liver* • Accentuated lobular pattern (slight) in ♂ (32%; 16/50 animals vs 6%; 3/50 in controls), and ♀ (20%; 10/50 animals vs 4%; 2/48 in controls)	B.6.5-04, 1995

Method	Results	Reference
	• ↑ Accentuated lobular pattern (any severity) in ♂ (68%; 34/50 animals vs 24%; 12/50 in controls), and ♀ (52%; 26/50 animals vs 15%; 7/48 in controls) • ↑ Focus of altered cells-eosinophilic, hepatocellular, focal or multifocal in ♂ (12%; 6/50 animals vs 6%; 3/50 in controls; n.s.) *Non-neoplastic changes: kidney* • ↑ Degeneration/regeneration tubule (very slight) in ♂ (70%; 35/50 animals vs 34%; 17/50 in controls) • ↑ Vacuolation decreased tubule (any severity) in ♂(100%; 50/50 animals vs 30%; 15/50 in controls) Systemic LOAEL = 250 mg/kg bw/day Systemic NOAEL < 250 mg/kg bw/day Critical effect at the LOAEL: changes in hepatocytes and kidney tubule morphology (♂,♀) **Target tissue/organ: liver and kidney**	
Two-generation At least 25 CD Sprague-Dawley rats/sex/dose OPP (purity 99.86%) 40, 140 and 490 mg/kg bw/day (actual doses: 35, 125, 457 mg/kg bw/day) for 2 generations	<u>Parental effects</u> *490 mg/kg bw/day: P generation* • ↑ Relative weight of ovaries in ♀ (33%, ndr) and of kidney in ♂(7%) • ↑ Incidence of renal calculi (13/35 vs 3/35 in controls) and haemorrhage (6/35 vs 0/35 in controls) in ♂ • ↑ Incidence of bladder calculi in ♂ (15/35 vs 9/35 in controls (46% vs 26%; n.s.) • ↑ Incidence of urinary bladder transitional cell hyperplasia in ♂ (23/35 vs 3/35 in controls) and ♀ (9/35 vs 1/35) • ↑ Incidence in bladder average no. cells/layer 81% in ♂ and 32% in ♀. ↑ of average microns at 10X 142% in ♂ and 50% in ♀ in bladder *490 mg/kg bw/day: F1 generation* • ↓ Absolute weight of liver (13%) and kidney (9%) in ♀ • ↑ Relative weight of testes (13%) and kidney (11%) in ♂ • ↑ Incidence of urinary bladder transitional cell hyperplasia in ♂ (15/35 vs 1/27 in controls) • ↓ Incidence of average no. cells/layer 10% in ♀ (n.s.; ndr) • ↑ Average microns at 10 X 62% in ♂ *140 mg/kg bw/day: P generation* • ↑ Relative weight of ovaries in ♀ (19%, n.s.) • ↑ Incidence of average no. cells/layer 29% in ♀. ↑ of average microns at 10 X 48% in ♂ and 51% in ♀ • ↑ Incidence of bladder calculi in ♂ (15/35 vs 9/35 in controls (46% vs 26%; n.s.) *140 mg/kg bw/day: F1 generation* • ↑ Absolute weight of liver (10.3%, ndr), kidney (9%, ndr) and testes (8%, ndr) in ♂ • ↓ Incidence of average no. cells/layer 26% in ♀ (ndr) *40 mg/kg bw/day: P generation* • ↑ Relative weight of ovaries in ♀ (29%, ndr)	B.6.6.1/01, 1990

Method	Results	Reference
	40 mg/kg bw/day: F1 generation • ↑ Absolute weight of kidney (7%, ndr) and testes (6%, ndr) in ♂ Parental LOAEL = 125 mg/kg bw/day Parental NOAEL = 35 mg/kg bw/day Critical effect at the LOAEL: bladder calculi (♂), urothelial hyperplasia (♂,♀) **Target organs/tissues: urinary bladder, urithelium and kidney**	
Two-generation 30 Albino CD Sprague-Dawley rats/sex/dose OPP (purity 99.7%) Dietary: 20, 100, 500 mg/kg bw/day (actual doses: 18/17, 93/92, 459/457 mg/kg bw/day for ♂/♀)	<u>Parental effects</u> *500 mg/kg bw/day: P generation* Urinary bladder: ↑ Incidence of histopathological alterations in ♂: [calculus (4/30 vs 0/30 in controls); chronic inflammation (13/30 vs 0/30 in controls); nodular/papillary (16/30 vs 1/30 in controls); simple hyperplasia (20/30 vs 1/30 in controls); ureter dilatation (4/30 vs 0/30 in controls) and hyperplasia (3/30 vs 0/30 in controls)] *500 mg/kg bw/day: F1 generation* Urinary bladder: ↑ Incidence of histopathological alterations in ♂: [calculus (4/30 vs 0/30 in controls); chronic inflammation (12/30 vs 0/30 in controls); nodular/papillary (19/30 vs 0/30 in controls), and simple hyperplasia (27/30 vs 0/30 in controls) Kidney: ↑ Incidence of kidneys debris in ♂ (4/30 vs 0/30 in controls); ↑ Incidence of calculi in ♂ (7/30 vs 0/30 in controls). Parental NOAEL: 100 mg/kg bw/day Parental LOAEL: 500 mg/kg bw/day **Target organ: urinary bladder**	B.6.6.1-02, 1995
Developmental toxicity 7 NZW female rabbit/dose OPP (purity 99.77%) Oral gavage: 0, 250, 500 and 750 mg/ kg bw/day from day 7 to 19 of gestation	<u>Maternal toxicity</u> *750 mg/kg bw/day: Gross pathology* Digestive tract haemorrhage, gaseous distension and erosions of the stomach, and decreased/soft ingesta of the gastrointestinal tract. Haemolysed blood in intestines. Pale kidneys. *750 mg/kg bw/day: Histopathology (not statistically analysed)* • Kidney: ↑ Autolysis (71%, 5/7 animals vs 0% in controls); ↑ Degeneration tubule(s), bilateral, diffuse, moderate (14%, 1/7 animals vs 0% in controls); ↑ Inflammation, bilateral, diffuse, moderate (14%, 1/7 animals vs 0% in controls) • Liver: ↑ Autolysis (71%, 5/7 animals vs 0% in controls) • Stomach: ↑ Erosion (s), mucosa, focal, slight (43%, 3/7 animals vs 0% in controls); ↑ Pigment-haematogenous-	B.6.6.2/03, 1991b

Method	Results	Reference
	increased, mucosa (43%, 3/7 animals vs 0% in controls) *500 mg/kg bw/day: Gross pathology* ↓ Body weight gain [GD 7-10 (101%)]; ↑ Kidney absolute/relative weight (15%, n.s./34%); pale kidneys. *500 mg/kg bw/day: Histopathology (Not statistically analysed)* • Kidney: ↑ autolysis (29%, 2/7 animals vs 0% in controls) • Liver: ↑ autolysis (29%, 2/7 animals vs 0% in controls) • Stomach: ↑ Pigment-haematogenous- increased, mucosa (29%, 2/7 animals vs 0% in controls) <u>250 mg/kg bw/day</u> *Gross pathology* • ↑ kidney relative weight (16%, n.s.). *Histopathology (Not statistically analysed)* • Kidney: ↑ autolysis (14%, 1/7 animals vs 0% in controls) • Liver: ↑ autolysis (14%, 1/7 animals vs 0% in controls) Maternal LOAEL = 250 mg/kg bw/ day Maternal NOAEL < 250 mg/kg bw/ day Critical effect at the LOAEL: alterations in the kidneys **Target tissue/organ: kidney**	
Developmental toxicity 16 to 24 NZW female rabbits/dose OPP (purity 99.77%) Oral gavage: 0, 25, 100, 250 mg/ kg bw/day from day 7 to 19 of gestation	<u>Maternal toxicity</u> 250 mg/kg bw/day: Gross pathology Ulceration and haemorrhage of the gastric mucosa, haemolysed blood within intestinal tract and decreased content and increased fluidity of ingesta *250 mg/kg bw/day: Histopathology (Not statistically analysed)* Kidney: ↑ degeneration, tubule(s); unilateral, focal: (4%, 1/24 animals vs 0% in controls); ↑ degeneration, tubule(s), bilateral, focal: (8%, 2/24 animals vs 0% in controls); ↑ degeneration, tubule(s), bilateral, multifocal (slight): (8%, 2/24 animals vs 0% in controls); ↑ degeneration, tubule(s bilateral, multifocal (moderate): (12.5%, 3/24 animals vs 0% in controls); ↑ inflammation, unilateral, focal: (4%, 1/24 animals vs 0% in controls); ↑ inflammation, bilateral, focal: (12.5%, 3/24 animals vs 0% in controls); ↑ inflammation, bilateral, multifocal (slight): (17%, 4/24 animals vs 0% in controls); ↑inflammation, pelvis, unilateral, focal (4%, 1/24 animals vs 0% in controls); ↑inflammation, pelvis, bilateral, focal (8%, 2/24 animals vs 0% in controls). Maternal LOAEL: 250 mg/kg bw/ day Maternal NOAEL: 100 mg/kg bw/ day Critical effect at the LOAEL: renal tubular degeneration	B.6.6.2/04, 1991c

Method	Results	Reference
	Target tissue/organ: kidney	
Sub chronic study into bladder effects 20 CDF[F-344]/BR male rats/dose OPP (purity 99.7%) Dietary: 0, 54, 224, and 684 mg/kg bw/day for 13 weeks	<u>684 mg/kg bw/day</u> *Bladder histopathology* • ↑ Simple hyperplasia (urothelium ≥ 4 cell layers) in 50% (5/10 animals vs 0% in controls) at week 4; in 30% (3/10 animals vs 0% in controls) at week 13, and in 10% (1/10 animals vs 0% in controls) at week 17 • ↑ Papillary/nodular hyperplasia (endo- or exophytic proliferations with a fibrovascular core) in 10% (1/10 animals vs 0% in controls) at week 13 • ↑ Occasional foci of one to a few necrotic or exfoliated cells (40%, 0% and 30% at week 4, 13 and 17, respectively vs 10%, 10%, 60% in controls) • ↑ Cobblestone appearance and/or more extensive and larger foci of necrosis/exfoliation (10%, 10% and 30% at week 4, 13 and 17, respectively; vs 0%, 10% and 10% in controls) • ↑ Extensive necrosis and appearance of rounded cells in addition to polygonal cells (30%, 20% and 10%, at week 4, 13 and 17, respectively vs 0%, 0% and 10% in controls) • ↑ Obvious piling up of round cells (hyperplasia), the cells usually having uniform and/or pleomorphic microvilli rather than microridges (20%, 70% and 30%, at week 4, 13 and 17, respectively vs 0%, 0% and 0% in controls) *Kidney histopathology* • ↑ Calcification at week 4 (10%, 1/10 animals vs 0% in controls; ndr); at week 13 (30%, 3/10 animals vs 0% in controls; ncdr) and at week 17 (40%, 4/10 animals vs 30% in controls) • ↑ Tubular proliferation at week 13 (30%, 3/10 animals vs 0% in controls); and at week 17 (10%, 1/10 animals vs 0% in controls) • ↑ Tubular dilatation at week 17 (20%, 2/10 animals vs 0% in controls) <u>224 mg/kg bw/day</u> • ↑ Occasional foci of one to a few necrotic or exfoliated cells (30%, 70% and 0% at week 4, 13 and 17, respectively vs 10% 10% and 60% in controls; n.s.) • ↑ Cobblestone appearance and/or more extensive and larger foci of necrosis/exfoliation (10%, 20% and 0% at week 4, 13 and 17, respectively; vs 0%, 10% and 10% in controls; n.s.) • ↑ Extensive necrosis and appearance of rounded cells in addition to polygonal cells (10%, 0% and 0%, at week 4, 13 and 17, respectively vs 0%, 0% and 10% in controls; n.s.) LOAEL = 684 mg/kg bw/day NOAEL = 224 mg/kg bw/day Critical effect at the LOAEL: kidney damage and morphological alterations of the urinary bladder epithelium (↑ mitogenesis, leading to a hyperplasia) (♂)	B.6.8.2-02, 1996a
	Target tissue/organ: kidney and bladder	

Method	Results	Reference
Sub chronic study (32P-postlabelling) 22 CDF[F-344]/BR male rats/dose OPP (purity 99.5%) Dietary: 0, 57, 285, 568, and 937 mg/kg bw/day for 13 weeks	*937 mg/kg bw/day: histopathology* • Bladder: ↑ Relative weight (35%); ↑ simple hyperplasia in 70% (7/10 animals vs 0% in controls) at week 13 • Kidney: ↑ relative weight (18%) *568 mg/kg bw/day: histopathology* • Bladder: ↑ relative weight (18%); ↑ simple hyperplasia in 20% (2/10 animals vs 0% in controls, n.s.) at week 13; ↑ occasional foci of one to a few necrotic or exfoliated cells (20% at week 13 vs 0% in controls; n.s.); ↑ extensive necrosis and appearance of rounded cells in addition to polygonal cells (20% at week 13 vs 0% in controls; n.s.); ↑ obvious piling up of round cells (hyperplasia), the cells usually having uniform and/or pleomorphic microvilli rather than microridges (60% at week 13 vs 0% in controls) • Kidney: ↑ relative weight (12%) LOAEL = 568 mg/kg bw/day NOAEL = 285 mg/kg bw/day Critical effect at the LOAEL: ↑ of mitotic activity and hyperplasia of the urothelium **Target tissue/organ: bladder**	B.6.8.2-03, 1996b
Pubertal development and thyroid function in intact juvenile/peripubertal female 15 Crl:CD(SD) female rats/dose OPP (99.9% purity) Oral gavage: 50, 250, 900 mg/kg bw/day from PND 22 to 42	<u>900 mg/kg bw/day: kidney histopathology (not statistically analysed)</u> • ↑ Dilatation tubule; focal/multifocal (very slight or slight) (79%, 11/14 animals vs 13%, 2/15 animals in controls) • Hypertrophy; collecting duct; multifocal (very slight) (21%, 3/14 animals vs 0% in controls) • Necrosis with accompanying inflammation; tubule; focal (slight) (14%, 2/14 animals vs 0% in controls) • Hyperplasia; epithelium; papilla; unilateral or bilateral; multifocal (very slight) (14%, 2/14 animals vs 0% in controls) <u>250 mg/kg bw/day: kidney histopathology (not statistically analysed)</u> • ↑ Dilatation tubule; focal/multifocal (very slight or slight) (27%, 4/15 animals vs 13%, 2/15 animals in controls) <u>50 mg/kg bw/day: kidney histopathology (not statistically analysed)</u> • ↑ Dilatation tubule; focal/multifocal (very slight or slight) (20%, 3/15 animals vs 13%, 2/15 animals in controls) **Target tissue/organ: kidney**	B.6.8.3.8, 2012
Pubertal development and thyroid function in intact juvenile/peripubertal male 15 Crl:CD(SD) male rats/dose	<u>900 mg/kg bw/day: kidney histopathology (not statistically analysed)</u> • ↑ Dilatation tubule; focal/multifocal (very slight or slight) (86%, 12/14 animals vs 27%, 4/15 animals in controls) • Hypertrophy; collecting duct; epithelium; focal/multifocal (very slight) (36%, 5/14 animals vs 0% in controls)	B.6.8.3.9, 2012

Method	Results	Reference
OPP (99.9% purity) Oral gavage: 50, 250, 900 mg/kg bw/day from PND 23 to 53	• Hyperplasia; epithelium; papilla; unilateral or bilateral; multifocal (very slight) (14%, 2/14 animals vs 0% in controls) **Target tissue/organ: kidney**	

Several repeated dose toxicity studies identified the urinary bladder as the main target organ of OPP. Some of the reported effects were abnormal growth in the bladder mucosa and increase of relative weight (study B.6.3.2-02); hyperplasia and masses (study B.6.5-02); calculi and urothelial hyperplasia (study B.6.6.1/01) and morphological alterations of the epithelium with increase of mitogenesis, leading to hyperplasia (studies B.6.8.2-02 and B.6.8.2-03 13).

Kidney was also a target organ in several repeated dose toxicity studies. The 13-week dietary study in rats (B.6.3.2-02) reported kidneys inflammation and increases of relative weight. The combined chronic toxicity/carcinogenicity in rats (study B.6.5-02) found calculus, hyperplasia, cysts, infarct, acute inflammation and papilla mineralization of kidney. The carcinogenicity study in mice (study B.6.5-04) demonstrated that OPP induced changes in kidney tubule morphology. Two developmental toxicity studies in rats (B.6.6.2/03 and B.6.6.2/04) reported renal tubular degeneration as main maternal toxicity effect. Finally, kidney damage was also demonstrated after OPP exposure in a sub-chronic study in rats (B.6.8.2-02) and in two mechanistic developmental toxicity studies (B.6.8.3.8 and B.6.8.3.9).

In addition, the carcinogenicity study in mice (B.6.5-04) highlighted liver as target organ toxicity reporting accentuated lobular pattern in several extensions (slight, moderate and severe) in both males and females and multifocal altered eosinophilic cells.

Comparison with the criteria

In all studies, effects on urinary bladder were reported at doses well above the limit for classification and therefore in this case, the conditions for classification are not met. In the case of kidney, three different developmental toxicity studies (B.6.6.2/03, B.6.6.2/04 and B.6.8.3.8) showed effects falling within the range of dose that could support a classification as Cat. 2. RAC notes that other repeated dose toxicity studies of longer duration (13-weeks and 2-years) (studies B.6.3.2-02 and B.6.5-02) did not reported histopathological alterations in kidney at doses (2798 and 402 mg/kg bw/day) quite higher than those reported in the two studies supporting classification (50 and 250 mg/kg bw/day). Thus, RAC considers that the kidney injuries reported are not sufficiently robust for supporting a classification as STOT RE for kidney.

In summary, **RAC supports the DS's proposal for no classification of OPP for STOT RE.**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

According to the DS, available *in vivo* germ and somatic cells mutagenicity assay data do not meet the criteria for classification but there is questionable data suggesting that OPP is able to cause clastogenicity *in vivo*. The DS initially proposed no classification of OPP for germ cell mutagenicity due to inconclusive data. However, during the consultation the applicant submitted two new *in vitro* assays (a reverse mutation assay with *Salmonella typhimurium* and *Escherichia coli* and an *in vitro* mammalian micronucleus assay in Chinese hamster V79 cells). Both assays yielded negative results. With these two new assays, the DS changed its position and considered that the genotoxicity of OPP is conclusively negative.

Comments received during consultation

A MSCA suggested that read-across and QSAR predictions could be used in a weight-of-evidence approach in order to clarify uncertainties about mutagenicity of OPP. DS replied that with the two new negative studies the genotoxicity assessment of OPP may be conducted.

Assessment and comparison with the classification criteria

In vitro studies

The table below summarises the *in vitro* genotoxicity studies with OPP. OPP showed negative results in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 in the presence and absence of metabolic activation (B.6.4.1.1-01). OPP was reported negative in *S. typhimurium* TA102 (B.6.4.1.1-02) and in *E. coli WP2* (B.6.4.1.1-03). A number of studies considered as supporting information due to method deficiencies confirmed these negative results (see Table 50 in CLH-report for details). OPP gave a slight positive increase in revertants in TA1535 without metabolic activation (B.6.4.1.1-04) but this result has not been reproduced in any of the additional supporting information studies (see Table 50 in CLH-report for details). Overall, based on the available information, it can be concluded that OPP is not mutagenic in bacteria gene mutation assays.

During the consultation, the applicant submitted a new reverse mutation assay with bacteria (*S. typhimurium* and *E. coli*) (table below). OPP did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used (with and without metabolic activation).

OPP did not cause gene mutations in the HGPRT forward mutation assay in CHO-WB1 cells in the presence or absence of metabolic activation (B.6.4.1.2-02), and it was concluded to be negative in two mouse lymphoma assays (B.6.4.1.2-03 and B.6.4.1.2-04). In both studies, OPP showed positive results at the highest dose in the presence of metabolic activation although high cytotoxicity was observed. According to the criteria from current OECD TG 490 (2016), positive results obtained with less than 10% total growth should not be considered positive, and therefore, the overall result is considered negative. Overall, based on the data available, OPP is not considered to be mutagenic in mammalian gene mutation assays.

A number of *in vitro* mammalian chromosome aberration tests were provided to evaluate the clastogenicity potential of OPP. Positive results in the presence of metabolic activation were obtained for OPP in CHO-K1 cells (B.6.4.1.3-05 and B.6.4.1.3-06). OPP also produced sister chromatid exchanges in the presence of metabolic activation. OPP was reported negative in the absence of metabolic activation in CHL cells (B.6.4.1.3-01 and B.6.4.1.3-03) or CHO-K1 cells (B.6.4.1.3-02 and B.6.4.1.3-04). Based on methodology deficiencies these studies are considered as supportive only. The OPP metabolites phenylhydroquinone (PHQ) and phenylbenzoquinone (PBQ) also produced chromosome aberrations in CHO-K1 cells in the presence and absence of metabolic activation (B.6.4.1.3-06). Overall, based on the data available, OPP induces chromosomal aberration and sister chromatid exchanges *in vitro*.

No evidence of an impact of OPP on DNA damage and repair was obtained in an *in vitro* UDS assay in rat hepatocytes (B.6.4.1.4-04). This study, however, was deemed supporting information based on method deficiencies. OPP showed a significant increase in DNA strand breaks in an *in vitro* comet assay using HepG2 cells (B.6.4.1.4-08). OPP did not cause DNA single strand breaks or 8-OH-dG formation in Chinese hamster V79 lung fibroblasts whereas the metabolite PHQ and, to a lesser extent, PBQ both produced a significant increase in both parameters (B.6.4.1.4-01 and B.6.4.1.4-06). A number of studies reported the DNA damage caused by PHQ and PBQ but not OPP (B.6.4.1.4-05 and B.6.4.1.4-06), all of them regarded as supporting information.

During the consultation, the applicant submitted a new *in vitro* mammalian micronucleus assay in Chinese Hamster V79 cells, where it did not induce structural and/or numerical chromosomal damage, with and without metabolic activation. Therefore, OPP is non-mutagenic with respect to clastogenicity and/or aneugenicity in this *in vitro* mammalian cell micronucleus test.

***Table**: Summary of mutagenicity/genotoxicity in vitro studies with OPP*

Method details	Results	Reference
Bacterial gene mutation (Ames test) Comparable to OECD TG 471 Deviations from the current OECD TG 471: characterisation and stability of test item not determined; TA102 or *E. Coli* WP2 uvrA not tested. GLP: Yes Vehicle: acetone *S. typhimurium*: TA98, TA100, TA1535, TA1537. 667 µg/plate (-S9) 1000 µg/plate (+S9) Study acceptable	**Negative (± S9)** Toxicity at the high dose levels tested in TA98 and TA100 in the first experiment	San and Springfield 1989a B.6.4.1.1-01
Bacterial gene mutation (Ames test) Deviations from the current OECD TG 471: characterisation and stability of test item not determined; only 4 strains used; data on concentration range or positive controls not reported. GLP: No Vehicle: DMSO *S. typhimurium*: TA97, TA98, TA100 and TA102- Rat S9 No information on test concentrations and no result table available Supporting information	**Negative (± S9)**	Pagano *et al*., 1988 B.6.4.1.1-02
Bacterial gene mutation (Ames test) Pre-guideline Deviations from the current OECD TG 471: characterisation and stability of test item not determined; data on test concentration range, positive controls not reported. GLP: No Vehicle not stated *S. typhimurium*: TA98, TA100, TA1535, TA1537 and TA1538 and *E. coli* WP2 hcr S9	**Negative (± S9)**	Shirasu *et al.,* 1978a B.6.4.1.1-03

Method details	Results	Reference
No information on test concentrations and no result table available Supporting information		
Bacterial gene mutation (Ames test) Comparable to OECD TG 471 (1997) Deviations from the current OECD TG: only 4 strains used. GLP: No OPP purity: 99.9%; Lot No.: MM09157 Vehicle: DMSO *S. typhimurium*: TA98, TA100, TA1535 and TA1537 3.3-200 µg/plate Rat and Hamster S9 Study acceptable	**Positive in TA1535 (-S9)** Slight positive increase in revertants at 100 µg/plate	Haworth *et al.*, 1983 B.6.4.1.1-04
HGPRT forward mutation assay GLP: Yes OPP purity: 99.9% CHO-WB1 cells 6.25-100 µg/mL (-S9) 12.5-115 µg/mL (+S9) Study acceptable	**Negative (± S9)** High cytotoxicity observed at high dose levels tested with and without metabolic activation	Brendler, 1992 B.6.4.1.2-02
TK+/- mutation assay in L5178Y cells (mouse lymphoma assay) Deviations from current OECD TG 490: characterisation of test item not determined; poor description of method (duration of exposure to test item, cell line origin); historical control data (HCD) not provided GLP: Yes L5178Y TK +/- Solvent: ethanol 18-44 µg/mL (-S9) 5-31 µg/mL (+S9) Study acceptable	**Negative (± S9)**	Harbell, 1989 B.6.4.1.2-03
TK+/- mutation assay in L5178Y cells (mouse lymphoma assay) Deviations from current OECD TG 490: HCD not provided GLP: No	**Negative (+S9)**	NTP, 1986 B.6.4.1.2-04

Method details	Results	Reference
OPP purity > 99% L5178Y TK +/- Solvent: water (-S9), DMSO (+S9) 20-60 µg/mL (-S9) 0.32-5 µg/mL (+S9) Study acceptable		
Mammalian chromosome aberration test Deviations from current OECD TG 473: no detailed experimental results data reported; only 100 metaphases scored; no metabolic activation; gaps not evaluated; no HCD available. GLP: no OPP: purity not stated Chinese hamster lung fibroblasts (CHL) Solvent: DMSO Up to 0.05 mg/mL 48h expression time Supporting information	**Negative (-S9)**	Ishidate *et al.,* 1984 B.6.4.1.3-01
Mammalian cell chromosome aberration test Deviations from current OECD TG 473: only 200 metaphases scored; gaps included in the chromosome aberration result; no HCD; positive control data provided; no metabolic activation used. GLP: no OPP: purity > 99% Chinese hamster ovary (CHO-K1) Solvent: DMSO 50-175 µg/mL 27h and 42h expression time Supporting information	**Positive (-S9)** for sister chromatid exchanges at 27h expression time **Positive chromosome aberration (-S9)** both at 27h and 42h expression time	Tayama-Nawai *et al.,* 1984 B.6.4.1.3-02
Mammalian cell chromosome aberration test Compilation of results for OPP GLP: on Supporting information OPP purity not stated	**Positive (-S9) CHO-K1 cells** **Negative (-S9) CHL cells**	Ishidate *et al.,* 1988 B.6.4.1.3-04

Method details	Results	Reference
Solvent: DMSO Chinese hamster lung fibroblasts (CHL) 100 µg/mL, 3h treatment Compilation of experimental results from publications OPP		
Mammalian cell chromosome aberration test Deviations from current OECD TG 473: only 100 metaphases scored; no experiments without S9 mix; no HCD. GLP: no Study acceptable OPP purity > 99% Phenylhydroquinone (PHQ): purity 98% Chinese hamster ovary K1 cells (CHO-K1) Experiment 1: OPP at various concentrations with S9 mix: 0, 25, 50, 75, 100, 125, 150 and 175 µg/mL. Experiment 2: 100 µg/mL at various % of S9. PHQ: -S9: 0-25 µg/mL; +S9 0-150 µg/mL	**OPP induced SCE's and chromosome aberrations (+S9)** **PHQ induced chromosome aberrations (+S9) and SCE's (± S9)**	Tayama *et al.,* 1989 B.6.4.1.3-05
Effects of cysteine and sulfhydryl compounds in the cytogenicity of OPP, PHQ and PBQ (mammalian cell chromosome aberration test) GLP: no OPP purity > 99% Phenylhydroquinone: purity > 98% Phenylbenzoquinone: purity > 98% Chinese hamster ovary K1 cells (CHO-K1) First experiment: +S9 OPP and PHQ with sulfhydryl compounds (cysteine and glutathione). Doses: 100 µg/mL Second experiment: -S9 OPP and PHQ with sulfhydryl compounds (cysteine and glutathione); doses: 10 mM (Cys or GHS); OPP: 0-150 µg/mL; PHQ: 0-600 µg/mL. Third experiment: ± S9 PBQ; doses: 0-10 µg/mL (-S9), 0-50 µg/mL (+S9) Study acceptable	Sulfhydryl compounds reduced markedly the incidence of SCE's of both OPP and PHQ. **OPP clastogenic (+S9)** PHQ and PBQ: cytotoxic and clastogenic ± S9	Tayama and Nakagawa, 1991 B.6.4.1.3-06
DNA single strand breaks and 8-OH-dG formation No guideline GLP: no	**OPP itself did not cause DNA single strand breaks**	Henschke *et al.,* 2000 B.6.4.1.4-01

Method details	Results	Reference
Purities not stated Chinese hamster V79 lung fibroblasts OPP: 50-400 µM PHQ: 25-45 µM PBQ: 20-30 µM Supporting information	or 8-OHdG formation. **PHQ and PBQ caused a significant increase in both parameters** at non-cytotoxic concentrations	
In vitro UDS assay Comparable to OECD TG 482 Deviations: Characterisation of test substance; material and methods poorly described; only 20 cells measured per concentration. GLP: no Rat F344 hepatocytes 100 nmol/mL Supporting information	**Negative** in UDS assay *in vitro*	Probst *et al.,* 1981 B.6.4.1.4-04
DNA reactivity in the presence of copper (II) ions No guidance GLP: no OPP Phenylbenzoquinone Phenylhydroquinone Purity not stated 32P-5'-End labelled DNA fragments from plasmid pbcNI Supporting information	**PHQ and PBQ plus H_2O_2 caused strong DNA damage**	Inoue *et al.*, 1990 B.6.4.1.4-05
DNA reactivity No guidance GLP: on Supporting information ortho-Phenylphenol Phenylbenzoquinone Phenylhydroquinone Purity not stated Supercoiled pUC18 plasmid DNA (form I) Linear form pUC18 plasmid DNA (form III)	**PHQ cleaves DNA in vitro** in a process that probably involves superoxide anion	Nagai *et al.*, 1990 B.6.4.1.4-06

Method details	Results	Reference
DNA reactivity by formation of 8-OHdG No guidance GLP: no Supporting information ortho-Phenylphenol Phenylbenzoquinone Phenylhydroquinone Purity not stated Calf thymus DNA Concentrations: 10-5 to 10-2 M CuCl2 and FeCl2 concentrations: 5 µM	**PHQ caused a dose dependent increase in 8-OHdG** EDTA (oxygen radical scavenger) inhibits the PHQ-induced formation of 8-OHdG. $CuCl_2$ had an effect in PHQ-dependent DNA cleavage	Nagai *et al.,* 1995 B.6.4.1.4-07
In vitro comet assay No guidance GLP: no OPP purity: 99% HepG2 cells Concentration 0-800 µM	**Significant increase of DNA strand breaks** at 400 and 800 µM	Li *et al.,* 2012 B.6.4.1.4-08
Reverse Mutation Assay using Bacteria (*S. typhimurium* and *E. coli*) OECD TG 471 GLP: yes OPP purity ≥ 99.8% Test system: *S. typhimurium*: TA98, TA100, TA1535, TA1537 and TA1538 Negative control: Distilled water Solvent: (DMSO) S9 = liver microsomal from Wistar rats Positive control (–S9): sodium azide for S. typhimurium TA100, TA1535 (10 µg/plate); 4-nitro-o-phenylene-diamine for S. typhimurium: TA98, TA1537 (10 µg/plate) for TA98 and 40 µg/plate for TA1537; methylmethanesulfonate for E. coli WP2 uvrA (pKM101) (1 µL/plate) Positive control (+S9): 2-aminoanthracene for S*. typhimurium*: TA98, TA100, TA1535, TA1537 and *E. coli* WP2 uvrA (pKM101) (2.5 µg/plate) for TA98, TA100, TA1535 and TA1537 (10 µg/plate) Experiment I: 3.16, 10.0, 31.6, 100, 316, 1000, and 2500 µg/plate	**Negative**	STUGC21AA1248-2 Study submitted by applicant during the consultation

Method details	Results	Reference
Experiment II: 1.0, 3.16, 10.0, 31.6, 100, 316, 1000 and 2500 µg/plate		
In vitro Mammalian Micronucleus Assay OECD TG 487 GLP: yes OPP purity ≥ 99.8% Test system Chinese hamster V79 cells Negative control: culture medium (MEM) Solvent: (DMSO) S9 = liver microsomal from Wistar rats Positive control (-S9): methylmethanesulfonate (25 µg/mL) Positive control (+S9): cyclophosphamide (2.5 µg/mL) Aneugenic control: colchicine (0.16 and 1.5 µg/mL) 0.039, 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, 5.0, 7.5 and 10 mM (with and without S9)	**Negative**	STUGC21AA1248-3 Study submitted by applicant during the consultation

In vivo

The table below summarises the genotoxicity/mutagenicity studies in mammalian somatic or germ cells *in vivo*. OPP was negative in a cytogenetic study in bone marrow cells of rats (B.6.4.2.1-01). However, this study can only be considered as supporting information. OPP gave conflicting results in two Comet assays *in vivo*. OPP did not show increases in tail length in hepatocytes and kidney cells when dosed orally (B.6.4.2.2-01). However, positive results were obtained in the stomach, liver, kidney, bladder and lung cells following the same experimental method (B.6.4.2.2-02). Based on the method deficiencies and deviations from guideline, both studies are regarded as supporting information only.

OPP and PHQ did not induce DNA damage in the bladder epithelial cells following intravesical injection into the bladder in a DNA alkaline elution assay *in vivo* (B.6.4.2.3-01 and B.6.4.2.3-02). The metabolite PBQ was shown to cause DNA damage in bladder epithelial cells in both studies.

OPP did not cause hyperploidy or ploidy in proliferating bladder epithelial cells (B.6.4.2.3-03). In the dominant lethal tests, OPP gave a negative result (B.6.4.3.1-01 and B.6.4.3.1-02). OPP was also negative in a sex-linked recessive lethal test in Drosophila (B.6.4.3.1-03). These studies are considered as supporting information.

Table: *Summary of the genotoxicity/mutagenicity studies in mammalian somatic or germ cells in vivo with OPP*

Method details	Results	Reference
Chromosome aberration *in vivo* Pre-guidance Deviations from OECD TG 475: purity not stated; no vehicle information; method poorly described (abstract); no individual data reported; no positive/negative control or HCD reported. GLP: no Male Wistar rats Bone marrow cells Oral daily doses of 50, 100, 200, 400 and 800 mg/kg bw for 5 days Single doses of 250, 500, 1000, 2000 and 4000 mg/kg bw Animals were killed 24h after treatment Supporting information	**Negative** in any tested doses (single or repeat exposure)	Shirasu *et al.,* 1978a B.6.4.2.1-01
Comet assay *in vivo* Pre-guidance Deviations from OECD TG 489: Only 4 animals used; duration of treatment is less than 2 days; no justification for using a viscous vehicle; number of total cells per organ is less than 150; no HCD reported. GLP: yes OPP: purity: 99.8% Male CD-1 mice Liver and kidney Oral gavage 0, 250, 2000 mg/kg bw Volume: 10 mL olive oil 4 mice/group Exposure duration: 3, 8 and 24 h. Animals were killed after treatment (3, 8 and 24 h) Supporting information	**Negative** No increases in tail length in hepatocytes and kidney cells Two animals died in the top dose group (2000 mg/kg bw)	B.6.4.2.2-01, 2000
Comet assay *in vivo* Pre-guidance Deviations from OECD TG 489: purity of test item not reported; no positive control used; duration of the treatment was less than 2 days; weight of animals not recorded; not enough time for the DNA to unwind; number of total cells per organ is less than 150.	**OPP induced DNA damage in the stomach, liver,**	Sasaki *et al.,* 1997 B.6.4.2.2-02

Method details	Results	Reference
GLP: no Purity not stated Male CD-1 mice Liver, lung, kidney, spleen, brain, bladder and bone marrow Volume: 10 mL olive oil 4 animals/group Exposure duration: 3, 8 and 24 h. Animals were killed after treatment (3, 8 and 24 h): damage in the stomach, liver, kidney, bladder and lung Supporting information	**kidney, bladder and lung**	
DNA alkaline elution assay *in vivo* No guideline GLP: no OPP: purity not stated 2,5-Dihydroxybiphenyl (PHQ): purity not stated 2-Phenyl-1,4-benzoquinone (PBQ): purity not stated Male F344/DuCrj rats Urinary bladder epithelium OPP Dose: 0.05% PHQ Dose: 0.05% PBQ Doses: 0.0005-0.1% Volume: 0.4 mL in 0.9% NaCl solution Intravesical injection into the bladder Exposure: 10 min Supporting information	**OPP and PHQ: Negative** **PBQ positive**	Morimoto *et al*., 1987 B.6.4.2.3-01
DNA alkaline elution assay *in vivo* No guideline GLP: no OPP: purity: 98% Phenylhydroquinone (PHQ): purity: 99% Phenylbenzoquinone (PBQ): purity: > 99% In situ study: OPP Dose: 0.05%; PHQ Dose: 0.05%; PBQ Doses: 0.0005-0.1%	**PBQ caused DNA damage** in the urinary bladder epithelium. **OPP and PHQ did not cause DNA damage** in the bladder epithelium	Morimoto *et al.,* 1989 B.6.4.2.3-02

Method details	Results	Reference
Volume: 0.4 mL Male F344/DuCrj rats Urinary bladder epithelium Vehicle: 0.9% NaCl solution Intravesical injection into the bladder Exposure: 10 min 5-10 animals/dose group Duration of exposure: 3-5 months Supporting information		
In vivo study for ploidy No guideline GLP: no OPP: purity not stated Urinary bladder epithelial cells 800, 2000, 4000, 8000 and 12500 ppm Oral diet Duration of exposure: 14 days Supporting information	**OPP did not cause hyperploidy or ploploidy** in proliferating bladder epithelial cells	Balakrishnan and Eastmond, 2003 B.6.4.2.3-03
Dominant Lethal test Pre-guidance Deviations from current OECD TG 478: purity of test substance not reported; exposure and mating did not cover an entire round of spermatogenesis; the MTD is not reported, no information on pregnant females/implantation/resorptions, etc. reported, no HCD reported. GLP: no OPP purity: 99.7% C3H male mice 0, 100 and 500 mg/kg bw Oral gavage Vehicle: water and 5% gam Arabic Volume: 2 mL/100 g bw 15 animals/dose group Duration of exposure: 5 days Supporting information	**Negative**	Kaneda *et al.,* 1978 B.6.4.3.1-01

Method details	Results	Reference
Dominant Lethal test Pre-guidance Deviations from current OECD TG 478: only abstract provided; no adequate study description. GLP: no OPP: purity not stated C3H male mice 0, 100 and 500 mg/kg bw Oral Duration of exposure: 5 days Supporting information	**Negative**	Shirasu *et al.,* 1978a B.6.4.3.1-02
Sex-linked recessive lethal test in Drosophila No guideline stated Deviations from OECD TG 477: no. of animals per dose group; no. of non-fertile males not indicated; no. of clusters of different sizes per male; no. of F2 cultures with progeny and number of chromosome lethal mutations at each germ cell stage not reported in the study. GLP: no OPP: purity: 99% Male and female Drosophila 250 ppm in feed or 500 ppm by injection Vehicle: 5% sucrose solution	**Negative**	NTP, 1986 B.6.4.3.1-03

Comparison with the criteria

Based on all the available genotoxicity data, the induction of chromosomal aberrations *in vitro* in mammalian cells (B.6.4.1.3-05 and B.6.4.1.3-06) cannot be confirmed with a reliable *in vivo* cytogenetic study as the *in vivo* chromosome aberration study provided (B.6.4.2.1-01) is deemed as supporting information only. The results from the two *in vivo* Comet assays (B.6.4.2.2-01 and B.6.4.2.2-02) are contradictory and both studies contain methodology deficiencies, hence deemed as supporting information only. Based on the weight of evidence from additional *in vivo* studies in germ cells, negative results were obtained in two dominant lethal tests (B.6.4.3.1-01 and B.6.4.3.1-02) although, based on deficient methods, they are also deemed as supporting information only. In conclusion, due to the high uncertainty of the available studies, the potential aneugenicity of OPP has not been suitably addressed with reliable *in vivo* cytogenetic studies.

Two DNA alkaline elution assay *in vivo* (B.6.4.2.3-01 and B.6.4.2.3-02) showed as after intravesical injection into the bladder OPP and its metabolite 2-phenylhydroquinone (PHQ) did not induce DNA damage; while the OPP metabolite 2-phenyl-1,4-benzoquinone (PBQ) did (tables 11 and 51 in the CLH report). On the other hand PHQ induced chromosome aberrations and sister chromatid exchanges in CHO cells in presence of S9 (B.6.4.1.3-05) and both (PHQ and PHQ) caused significant increase in DNA single strand breaks and 8-hydroxy guanine formation in V79

lung fibroblasts (B.6.4.1.4-01) (tables 10 and 50 in the CLH report). Overall, RAC notes that the OPP metabolites PHQ and PBQ have shown a certain capability to alter DNA *in vitro*, but it has been confirmed *in vivo* only for PHQ, although using a route of administration physiologically non-relevant (intravesical injection into the bladder).

No human data are available for OPP, hence a classification as Category 1A is not warranted. The classification as mutagen 1B must rely on positive *in vivo* results with heritable germ or somatic cells. However, such studies are negatives and of low reliability and therefore do not warrant classification within category 1B. The classification in Category 2 could be based on positive *in vitro* results that were supported by positive somatic cell mutagenicity tests *in vivo*. Such *in vivo* support has not been met due to low reliability of the *in vivo* data.

In summary, the positive *in vitro* results pose some concern but the conditions for classification have not been met and **RAC supports the DS's proposal for no classification of OPP for germ cell mutagenicity.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

DS proposed the classification of OPP as Carc. 2; H351, suspected of causing cancer, based on the urinary bladder tumours detected in rats.

Comments received during consultation

A manufacturer/company submitted comments stating that the mode of action for the urinary bladder tumours detected in rats is certainly not relevant for humans at doses that are associated with the proposed uses of OPP. DS replied that a more solid argumentation would be necessary for ruling out the relevance of this mode of action for humans. For example, some of the lacking data are temporal association of events, analysis of plausibility, analysis of potential alternative modes of action, etc.

One MSCA supported the DS's proposal for classification of OPP as Carc. 2.

Assessment and comparison with the classification criteria

The CLH report contains three dietary studies in rats and one in mice; additionally a 2-year dermal study in mice is available as well.

Due to the high number of deficiencies found in the first study (B.6.5/01) (see more details in Table 53 of CLH report), only limited information about long-term effects and carcinogenicity can be derived from it. Nevertheless, based on histopathological findings in kidney (tubular dilatation), decreased body weight and increased in testes weight; the NOAEL was considered to be 2000 ppm (≈100-200 mg/kg bw/day).

The second study is a combined chronic toxicity/carcinogenicity study (B.6.5/02), in which systemic toxicity was manifested as decreased body weight at mid and high doses for both sexes during the entire treatment period (see Table 53 of CLH report for a detailed description of the systemic toxicity). There was an increase in urinary bladder hyperplasia at 12 and 24 months in high dose males and at 24 months in high dose females, along with an increase in congestion, haemorrhage, mineralisation and necrosis (see table in STOT RE section). Non-neoplastic findings consisted of increased incidence of calculi in the kidneys in high dose males and in the urinary

bladder at 12 and 24 months, respectively. High dose males and females also had an increase in cysts of the kidneys at 24 months. High dose females had an increase in hyperplasia of the kidney along with increase infarct, acute inflammation and mineralisation of the kidney (see table in STOT RE section).

In addition to the non-neoplastic histopathological injuries in urinary bladder summarised in the STOT RE section above, in male rats there was an increased incidence of urinary bladder papillomas and transitional cell carcinomas at 8000 ppm.

***Table**: Neoplastic histopathological findings in urinary bladder of males in the combined chronic toxicity/carcinogenicity study in rats (B.6.5/02).*

	0 mg/kg bw/day	402 mg/kg bw/day
Transitional cell carcinomas in at 24 months	0/50	34/50
Papillomas 12 months	0/20	6/20
Papillomas at 24 months	0/50	6/50

The third study (B.6.5/03) is a published report. OPP was mixed with the diet at concentrations of 6500, 12500 or 25000 ppm (269, 531 or 1140 mg/kg bw/day) to groups of 20-24 male F344 rats for 91 weeks to evaluate the carcinogenicity towards the urinary tract. Under the conditions of this study, OPP was carcinogenic in male F344 rats, causing urinary bladder tumours (papilloma and carcinoma) at 12500 ppm (see table below). Hyperplasia and calculi were also observed at 12500 and 25000 ppm. Increased mortality, decreased body weight and nephrotoxicity (gross haematuria) was also found at doses of 12500 and 25000 ppm.

***Table:** Hyperplastic or neoplastic lesions in urinary bladder in B.6.5/03 study. * = Statistically significant (p < 0.001)*

OPP [ppm]	**Examined rats**	**Number of rats with:**			
		Tumours	**Hyperplasia**	**Papilloma**	**Carcinoma**
0	24	0	0	0	0
6250	20	0	2	0	0
12500	24	23*	0	3	20
25000	23	4	7	2	2

Additionally to the findings reported in these three rat studies, urothelial hyperplasia of the urothelium was detected in male rats in the first generational study (B.6.6.1-01) and in males and females in the second generational study (B.6.6.1-02) (see STOT RE see table in STOT RE section).

In a dietary study (B.6.5-04), mice were administered OPP for 24 months, systemic toxicity was noted as decreased body weight gain throughout the study, an increase in absolute and relative liver weights at 12 and 24 months in all treated animals, a dose-related decrease of micro-vacuolation in the tubular epithelial cells of the kidney cortex, and a decrease in the incidence and severity of degeneration/regeneration of their tubules at 12 and 24 months in males. Systemic toxicity in this study is summarised in the STOT RE section, see also table 53 of the CLH report for a detailed description of the non-neoplastic toxicity.

Mice did not develop any treatment-related effects in the urinary bladder. An increased incidence of liver adenoma, carcinoma and hepatoblastoma was observed in male mice at 500 mg/kg bw/day and 1000 mg/kg bw/day. No concurrent HCD for these tumours was provided.

***Table**: Tumour incidences in the dietary study in mouse (B.6.5-04). * Statistically different from control mean by χ^2 pairwise test, α=0.10, two-sided, α=0.05, one-sided*

	Males				Females			
OPP [mg/kg bw/day]:	**0**	**250**	**500**	**1000**	**0**	**250**	**500**	**1000**
Number of mice examined	50	50	50	50	48	50	50	50
Type of tumour								
Hepatocellular adenoma (1)	27	33	40*	41*	13	14	17	19
Hepatocellular carcinoma (2)	11	5	14	12	2	8	6	5
Malignant hepatoblastoma (3)	0	2	6	3	0	0	0	0
Combined (2) + (3)	11	7	19	15	2	8	6	5
Combined (1) + (2) + (3)	32	36	45*	43*	15	22	23	24

Statistically significant increase of liver adenomas was described in the 2-year mice study (B.6.5-04) for mid and high dose male groups (80% and 82%, respectively), compared to controls (54%) (table above). Although suitable HCD were not available at the performing laboratory for the B6C3F1 strain of mouse, the NTP has extensive HCD for this strain of mouse during period 1990-1997[2]. The incidence of liver adenomas described in mid and high dose groups exceed the overall historical mean and range provided by NTP (mean= 29.4; range: 4-60%).

On the other hand, the incidences of hepatocellular carcinomas in 2-year male mice study were similar to controls and did not show a dose-response pattern (24%, 28%, 10% and 22% for high, mid, low and control groups, respectively). The incidences in treated groups were within the range of HCD provided by NTP (mean=17.9; range: 6-29%).

Additionally, the incidence of malignant hepatoblastoma did not display statistically significance and was not dose-related (6%, 12%, 4% and 0%, for high, mid, low and control male groups, respectively). However, the incidences in treated groups exceed the overall historical mean and range provided by NTP (mean= 0; range: 0%).

The study B.6.5-04 combined these three tumour types to show a statistically significant increase in mid and high dose groups (90% and 86%, respectively) compared to controls (64%) RAC notes that hepatoblastomas originate from a different cell population and adding these tumours to hepatocellular adenomas and carcinomas is not an appropriate method to determine statistical significance of liver tumours.

On the other hand, liver neoplasms incidences in female mice did not display statistically significant results, the neoplasms were within the range of HCD reported from NTP carcinogenesis program, and did not show dose-relationship.

Overall, there is evidence of association between OPP exposure and benign neoplasms, but there is no evidence of liver carcinogenicity after OPP administration.

A 2-year dermal study in mice (B.6.5/01)

The study B.6.5/01 was performed in mice to determine whether OPP was a carcinogen for skin or a tumour promoter in a two-stage initiation/promotion skin mode (initiation/promotion with DMBA). Under the conditions of this study, there was no evidence of carcinogenicity in male or female Swiss CD-1 mice when OPP was administered alone or as a promoter. However, OPP caused non-neoplastic lesions, which included ulceration, inflammation, and hyperkeratosis, at the site of application.

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[2] Haseman *et al.*, Spontaneous Neoplasm Incidences in Fischer 344 Rats and B6C3F, Mice in Two-Year Carcinogenicity Studies: A National Toxicology Program Update*Toxicol Pathol., 1998, 26(3):428-41.

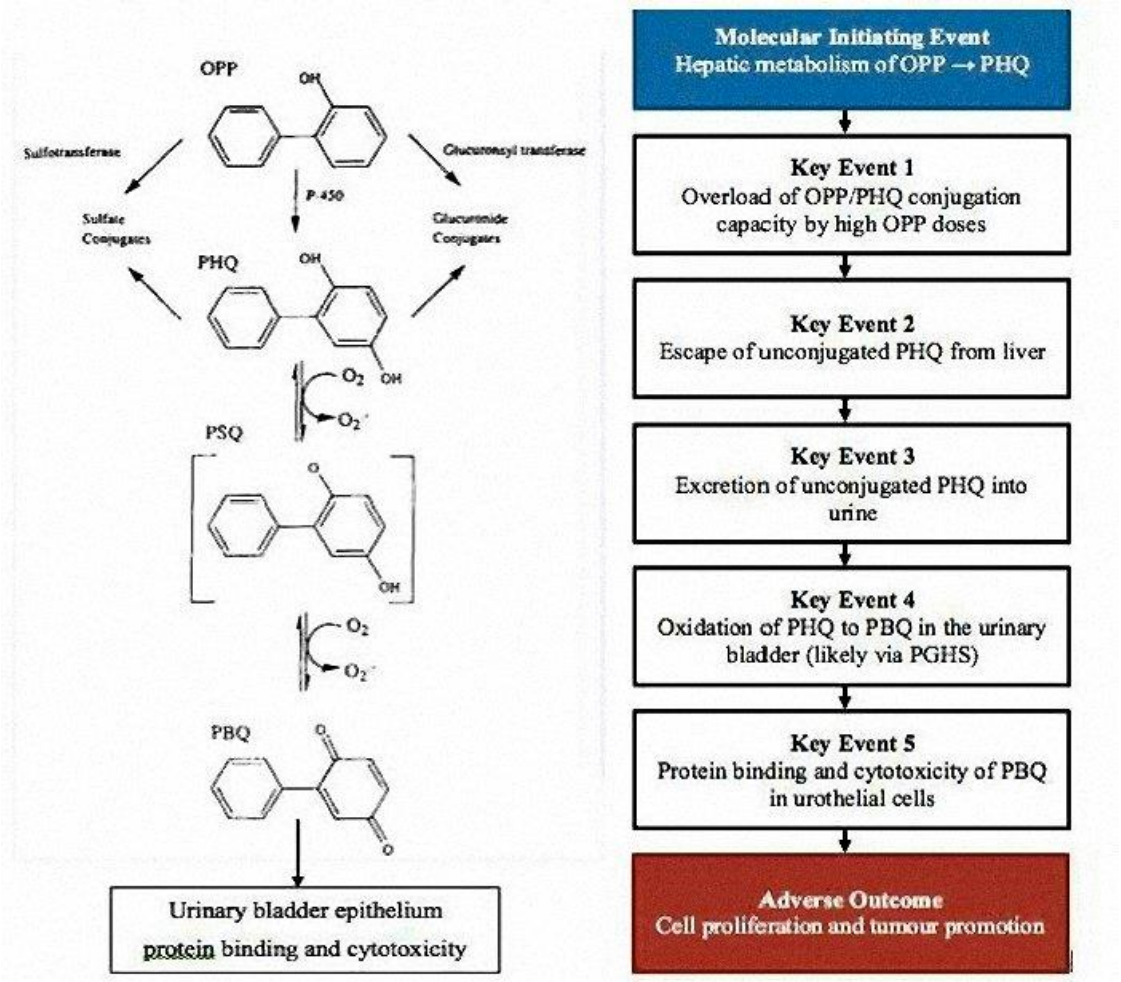
Mode of action of the urinary bladder tumours

Table 55 in CLH report presents a number of mechanistic studies for determining the mode of action of the urinary bladder tumours induced by OPP. The main conclusions from these studies are:

- The tumorigenic potential of OPP was enhanced by co-administration of sodium bicarbonate as an alkalinising agent (B.6.8.2-09 and B.6.8.2-12). Besides pH, morphological changes of the bladder epithelium were also enhanced by reduced urinary osmolality (B.6.8.2-10) and by increased Na^+ concentration (6.8.2-04).
- Increased DNA synthesis in the bladder epithelium could be detected following OPP (B.6.8.2-17) administration to rats. This mitotic activity could be clearly associated with morphological changes of the bladder epithelium (B.6.8.2-20).
- In the 13-week study (B.6.8.2-02) in which bromodeoxyuridine (BrdU) was used for assessment of mitotic activity, kidney damage and mitogenesis of the urinary bladder epithelium leading to hyperplasia were seen in male rats.
- No DNA-adducts could be detected after treating rats with OPP (B.6.8.2-11). This is in accordance with observations made in a sub chronic rat study (B.6.8.2-03) (see table in STOT RE section), suggesting that bladder carcinogenesis is likely mediated by a cytotoxic rather than a genotoxic effect.
- *In vivo* binding of OPP to cellular macromolecules was described in one study, without specifying the nature of these macromolecules (B.6.8.2-21). This study also describes a non-linear increase in this macromolecular binding *in vivo* and *in vitro*, which may be caused by the saturation of detoxification pathways.
- OPP is oxidised to PHQ, and PHQ is oxidised to PBQ by cytochrome P-450. PBQ is reduced back to PHQ by cytochrome P-450 reductase (B.6.8.2-16).
- OPP or its metabolites form protein adducts in the bladder, whereas DNA adducts could not be found (B.6.8.2-22). The study also showed that the bladder has a greater tendency for protein adduct formation than liver or GSH involvement of PGHS, an enzyme known to oxidise phenolic compounds to more reactive quinone species. This enzyme is highly expressed in the urinary bladder.
- OPP stimulated PGHS-dependent cyclooxygenase activity *in vitro* and were oxidised in the presence of the enzyme. OPP, PHQ and PBQ inhibited PGHS at higher concentrations (B.6.8.2-19). The latter finding might explain the observations made in the 91-week-study on rats (B.6.5-03), an increased incidence of bladder tumours was seen at dietary OPP levels of 12500 ppm, but not at 25000 ppm (see table in this section).
- OPP treatment led to GSH depletion and eosinophilic degeneration of centrilobular hepatocytes. Inhibition of GSH synthesis aggravated hepatotoxicity of OPP. In addition, PBQ induced hepatic and renal damage, while PHQ produce no significant adverse effects (B.6.8.2-14 and B.6.8.2-18). OPP cytotoxicity is enhanced by monooxygenase inhibition and GSH depletion. PHQ-induced cell death can be inhibited by sulphydryl compounds (B.6.8.2-15).
- An investigation about the tumour-promoting properties of OPP after initiation with BBN shown that OPP (20000 ppm) alone did not cause neoplasia in the urothelium with or without initiation with BBN (B.6.8.2-08).
- OPP caused a dose-dependent increase in agglutinability of bladder epithelial cells by Concanavalin A, indicative of carcinogenic potential (B.6.8.2-13).

In conclusion, a non-genotoxic MoA for tumorigenesis in rat urinary bladders is likely. The mode of action and adverse outcome pathway is depicted in the figure below. This mechanism could involve chronic irritation of the epithelium by a combination of high pH, reduced urinary osmolality, high sodium ion concentration and/or high concentration of free metabolites after excessive dose of OPP exposure; followed by regenerative hyperplasia and eventually tumours.

Metabolism studies have shown than OPP in rodents is rapidly converted into conjugates, which are eliminated via urine, the same can be applied to humans (B.6.1.2-01 and B.6.1.2-02). *In vitro* genotoxicity studies performed with main OPP metabolites, PHQ and PBQ, showed positive results for oxidative damage and cytotoxicity. OPP caused protein-binding (non-linear increase) and cell proliferation in bladder epithelial cells from treated male F344 rats supporting a non-genotoxic mechanism for bladder tumour formation from treated male F344 rats and a threshold mechanism is proposed. A contributory role of oxidative DNA damage cannot be excluded but this would not be expected to occur at low dose levels.



Proposed mode of action and adverse outcome pathway for bladder carcinogenesis induced by OPP. OPP = 2-phenylphenol. PHQ = phenylhydroquinone. PBQ = phenylbenzoquinone. Figure taken from CLH-report.

Mode of action of the hepatic tumours

Table 55 in the CLH report presents mechanistic studies for determining the mode of action of the hepatic tumours induced by OPP. The main conclusions from these studies are:

- among the nuclear receptors AhR, CAR, PXR, and PPARα, only PPARα mediated gene expression was elevated following OPP exposure in mice (B.6.8.2-23);
- OPP leads to transactivation of the human PXR, but not of the murine PXR (B.6.8.2/24).

The MoA for liver neoplasms in B6C3F1 mice induced after OPP treatment seems to involve PPARα-dependent rodent liver tumour response as noted by the increased expression of the cyp4a10 PPARα-response gene (B.6.8.2-23). It is known that the PPAR-dependent rodent liver

tumour response is "unlikely to be relevant" to humans, as explicitly mentioned in the OECD guidance for analysis and evaluation of chronic toxicity and carcinogenicity studies. However, RAC notes that more experimental evidence is needed to rule out other possible modes of action relevant for humans.

Comparison with the criteria

RAC notes that liver benign adenomas are of low concern because it seems that they do not progress to carcinomas.

Industry has provided comments suggesting that the mode of action of the malign urinary bladder tumours are specific to the rat. The main arguments to support this statement are:

- OPP has been shown to act as a tumour promoter only, not as a tumour initiator (B.6.8.2-08);
- Protein-, but no DNA-binding of OPP metabolites has been detected in the urinary bladder (B.6.8.2-03);
- Seemingly only the urinary bladder and a single sex is affected, thus the evidence for carcinogenicity is only "limited", following the definition given in Annex I, Section 3.6.2.2.3 of the CLP Regulation;
- Sulphate and glucuronide conjugation of OPP and PHQ prevents further oxidation to the ultimate protein-reactive and cytotoxic molecule PBQ. The conjugates are excreted via urine without undergoing toxification. High systemic OPP doses are required to elicit Key Event 1 by overloading the conjugation capacity of the liver. Key Event 5 (macromolecular binding) was only seen in rats at oral doses of at least 200 mg OPP/kg bw (B.6.8.2-21);
- Increased urinary pH and sodium concentration promote bladder neoplastic effects by OPP (B.6.8.2-10 and B.6.8.2-04). The pH, sodium concentration and osmolality of human urine are lower than in rat;
- Urinary bladder tumours only appeared in rats.

However, RAC notes that:

- PBQ (an OPP metabolite present in rats) caused DNA damage in the urinary bladder epithelium (B.6.4.2.3-01) (see table in STOT RE section)
- Although neoplasias have not been detected in the urinary bladder of female rats, hyperplasias of the urothelium have (B.6.6.1-01 and B.6.6.1-02) (see table in STOT RE section)
- The fact that high systemic OPP doses are necessary to elicit this effect bears no relevance as to the specificity of this mode of action to the rat. Moreover, human absorption and distribution of OPP is similar to that of rats (B.6.1.2-01 and B.6.1.2-02)
- The fact that the pH and sodium concentration of human urine is lower than in rat urine does not make the suggested mode of action rat specific

In conclusion, **even though a quite plausible non-genotoxic mechanism has been postulated, the human relevance of this mode of action cannot be ruled out.**

According to the criteria contained in the CLP Regulation, and in the absence of human studies, to classify a substance as a carcinogen in category 1, sufficient evidence of carcinogenicity in animal studies is necessary. Such sufficient evidence is reached when a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. These conditions are not met by the bladder tumours since they appear only in rats and only in males and therefore Cat. 1B is not warranted.

Given that RAC has not ruled out the relevance of bladder tumours for humans, it seems that evidence of carcinogenicity are limited: the data suggest a carcinogenic effect but are limited because the evidence of carcinogenicity is restricted to a single experiment and moreover there are unresolved questions regarding the interpretation of the studies. Thus, the conditions for classification as Cat. 2 have been met.

In conclusion**, RAC supports the DS's proposal for classification of OPP as Carc. 2; H351, suspected of causing cancer**, based on the urinary bladder tumours detected in rats.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

DS proposed no classification of OPP for fertility and sexual function based on the lack of effects on two reliable 2-generation reproductive toxicity studies.

DS reported discrepancies in the interpretation of a developmental toxicity study in rabbits. In this study, some authors interpreted that significant increase in the incidence of resorptions at 100 mg/kg bw/day occurred in absence of significant maternal toxicity, while other authors interpreted that such resorptions were concurrent with maternal toxicity. Finally, the DS considered the available information not to be sufficiently convincing for supporting a classification of OPP for developmental toxicity.

DS proposed no classification of OPP for effects or on via lactation based on lack of evidence for supporting such classification.

Comments received during consultation

A company manufacturer commented that the published re-evaluation of reprotoxicity (Kwok and Silva, 2013; B.6.6.2-06) contains factual errors and should not overturn previous EU evaluations of the available studies. The company manufacturer commented about another expert assessment contradicting the meta-analysis based on a statistical artefact without toxicological significance. According to this expert statement, the only new element of this B.6.6.2-06 review is the analysis of the data of the B.6.6.2/04 rabbit developmental toxicity study. Furthermore, according to this expert position paper, Kwok and Silva (2013) make several unsubstantiated speculations, in particular that post-implantation losses may have been underestimated in several studies by misclassification, either as pre-implantation losses or as non-pregnancies. Finally, this expert statement concludes that there are no new concerns about the OPP developmental toxicity and that the classification is not warranted.

A MSCA demanded clearer elaboration about the relevance of Kwok and Silva (2013) in the weight of evidence about the developmental toxicity. The DS replied that they did not regard adverse the increases since there was no dose-dependency, the occurrence was also high in concurrent controls and there was not an impact in other indices such as the number of foetuses per litter.

Assessment and comparison with the classification criteria

Fertility and sexual function

The reproductive toxicity of OPP was assessed in two 2-generation rat reproductive studies.

Two-generation study in rats (B.6.6.1-01)

In the first two-generation study (B.6.6.1-01), rats were administered OPP at doses of 0, 40, 140, and 490 mg/kg bw/day (actual doses of 0, 35, 125, and 457 mg/kg bw/day) in the diet. The main finding after OPP administration at the highest dose was the body weight depression that occurred in parents from both generations during pre-mating, gestation and lactation phases. The table in STOT RE section summarises the parental toxicity, see also table 57 in CLH report for a more detailed description of such parental toxicity.

Regarding reproductive parameters, no differences were detected between treated groups and controls in both generations. Only female fertility index was increased in low and mid dose groups (68% and 64%, respectively) in F1b generation compared with controls (32%) (table below). However, this increase in the fertility index is considered an artefact due to the extremely low fertility index for the control group (32%), and may have been due to the older age of the animals (approximately nine months).

The reproductive parameters evaluated in this study were seemingly not affected up to a dose of 457 mg/kg bw/day; however, the study lacks much of the information required for this assessment. Moreover, the information that it contains may not be completely reliable. As Kwok and Silva point out in 2013 (B.6.6.2-06), some dams were not co-housed with a male for long enough and/or were noted as having a sperm plug in their bedding or even vagina but not classified as having mated despite finding these plugs.

There was an increase in the incidence of pelvis dilatation in pups (21 days and older) in dosed groups, but this effect cannot be attributed to OPP administration. The incidence was increased in a dose-related manner in F1a females, but not in F1b/F2a/F2b females or males.

Two-generation study in rats (B.6.6.1-02)

In the second two-generation study (B.6.6.1-02), rats were exposed to nominal doses of 0, 20, 100 and 500 mg OPP/kg bw/day (actual doses: 18/17, 93/92, 459/457 mg/kg bw/day for males/females). Toxicological effects were manifested only at the 500 mg/kg bw/day dose level. Parents showed reduced body weight during pre-mating, gestation and lactation. The target organ was the urinary bladder. Males of both generations dosed with 500 mg/kg bw/day showed an increased incidence of calculi present in this organ. Microscopically, chronic inflammation and hyperplasia (simple and nodular) could be observed with increased incidence in males of this dosing group. The relative testis weight increased statistically in F1 males. OPP did not exert manifested toxicity in the offspring, apart from a statistical body weight depression in F1 pups around the weaning period and earlier, from day 14 onwards in case of F2 offspring. The table in STOT RE section summarises the parental toxicity, see also table 57 in CLH report for a more detailed description of such parental toxicity.

No effect on reproductive parameters was seen at any dose level. However, some parameters were not evaluated, such as sperm parameters and sexual maturation milestones. Another problem with reproductive parameters is the fact that the lowest ability to procreate (as indicated by the fertility index) was seen in F2a and F2b controls (as indicated in B.6.6.2-06); since this often led to fertility index increases with increasing dose. When evaluating both the fecundity and fertility indices, it appeared that the control group did not function as such. When this occurs, the potential for identification of true effects induced by treatments is limited.

***Table**: Summary of animal studies on adverse effects on sexual function and fertility with OPP. Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr).*

Method	Results	Reference
Two-generation At least 25 CD Sprague-Dawley rats/sex/dose OPP (purity 99.86%) 40, 140 and 490 mg/kg bw/day (actual doses: 35, 125, 457 mg/kg bw/day) for 2 generations	**PARENTAL TOXICITY** See a summary in STOT RE and more detailed information in table 57 of CLH report **REPRODUCTIVE PARAMETERS** <u>P and F1:</u> *490 mg/kg bw/day* • ↑ ♀ fertility index (47%, ndr; n.s.) during F1b generation vs 32% in controls *140 mg/kg bw/day* • ↑ ♀ fertility index (64%, ndr) during F1b generation vs 32% in controls *40 mg/kg bw/day* • ↑ ♀ fertility index (68%, ndr) during F1b generation vs 32% in controls **LITTER DATA** <u>490 mg/kg bw/day</u> *P→F1A and F1B:* P→F1A: ↑ live birth index (12%) (100% vs 88% in controls) P→F1B: ↓ Pup body weight (day 14 (13%) and day 21 (18.4%) postpartum) *F1→F2A and F2B* F1→F2A: ↓ Pup body weight (day 14 (6%) and day 21 (12%) postpartum) F1→F2B: ↓ Pup body weight [day 21 (12%) postpartum] <u>140 mg/kg bw/day</u> P→F1A: ↑ live birth index (97% vs 88% in controls)	B.6.6.1/01, 1990
Two-generation 30 Albino CD Sprague-Dawley rats/sex/dose OPP (purity 99.7%) Dietary: 20, 100, 500 mg/kg bw/day (actual doses: 18/17, 93/92, 459/457 mg/kg bw/day for ♂/♀)	**PARENTAL TOXICITY** See a summary in STOT RE and more detailed information in table 57 of CLH report **REPRODUCTIVE PARAMETERS** <u>P, F1 and F2</u> *500 mg/kg bw/day* • ↑ ♀ fertility index (96.6%) during F2b generation vs 66.7% in controls Gestation • ↑ food consumption in F1a throughout days 0-6 (11%) • ↑ food consumption in F1b throughout days 13-20 (12%) • ↑ food consumption in F2b throughout days 13-20 (11%)	B.6.6.1-02, 1995

Method	Results	Reference
	Lactation • ↑ food consumption in F1a throughout days 7-14 (17%) and 14-21 (12%) • ↑ food consumption in F1b throughout days 6-13 (22%) and 13-20 (12%) • ↑ food consumption in F2a during days 14-21 (12%) • ↑ food consumption in F2b during days 14-21 (11%) *100 mg/kg bw/day* • ↑ ♀ fertility index (81.5%, ns) during F2b generation vs 66.7% in controls Gestation • ↑ food consumption in F2b throughout days 13-20 (9%) *Lactation* • ↑ food consumption in F2a throughout days 14-21 (7%) *20 mg/kg bw/day* • ↑ ♀ fertility index (67.9%, ns) during F2b generation vs 66.7% in controls **LITTER DATA** 500 mg/kg bw/day *P→F1A and F1B* P→F1A: ↓ Pup body weight (day 21 (12%)) P→F1B: ↓ Pup body weight (day 21 (10%)) *F1→F2A and F2B* F1→F2A: ↓ Pup bw. (day 14 (6%) and day 21 (11%)) F1→F2B: ↓ Pup bw. (day 14 (7%) and day 21 (12%))	

Developmental toxicity

The CLH report contains five developmental toxicity studies performed with OPP, two in rabbits, two in rats and one in mouse.

Developmental toxicity study in rats (B.6.6.2/01)

In the first rat developmental toxicity study (B.6.6.2/01), OPP was administered to pregnant rats at doses of 0, 150, 300, 600 and 1200 mg/kg bw/day during the organogenesis period. At 1200 mg/kg bw/day, there was excessive mortality (10 of 11) after 3-9 days of treatment (table below), but no necropsy data is available in this study. Dams developed ataxia for several hours after substance administration at doses of 300 mg/kg bw/day or higher. In addition, females treated with at least 300 mg/kg bw/day showed a noticeable body weight gain depression (table below).

Effects to foetuses from OPP exposure *in utero* in the 300 mg/kg bw/day group appeared as increased incidence of foetal malformations (i.e. cranial or sacral meningocele, hydronephrosis, and diaphragmatic hernia). Effects to foetuses from 600 mg/kg bw/day OPP exposure group appeared as an increased incidence of resorptions and reduced foetal body weights (both sexes). In both cases, there were no statistically significant differences between the incidences of such foetal malformations in control and exposed animals (table below). Overall, RAC notes that the lack of statistical significance of these effects preclude that could be used for substantiating an eventual classification for developmental toxicity.

Developmental toxicity study in rats (B.6.6.2/02)

In the second rat developmental toxicity study (B.6.6.2/02), pregnant rats were dosed with 0, 100, 300 and 700 mg/kg bw/day. Results were not recorded for two control dams and four dams at 700 mg/kg bw/day because they were given the wrong dose, were not pregnant, or delivered early. One dam died at 700 mg/kg bw/day due to dosing error but there were no treatment-related deaths (see table below).

Rats dosed with 700 mg/kg bw/day experienced a statistically body weight, body weight gain and food consumption decrease, especially during the first 6-10 days of treatment (see table below). After the scheduled sacrifice, decreases in absolute liver weights were observed during necropsy (see table below).

There were no effects on foetal developmental parameters and no external or visceral effects were observed. Delayed ossification in sternebrae and skull were statistically significantly increased at 700 mg/kg bw/day. In particular, delayed ossification of the sternebrae was observed in 3% of foetuses and 30% of litters at 700 mg/kg bw/day and was outside the HCD (5% foetuses and 28% litters). Overall, RAC notes statistically significant alterations in delayed ossification but concurrently with reductions in body weight gain of 64% between days 6-9.

A possible statistically significant increase in pre-implantation loss at 700 mg/kg bw/day has been described by Kwok and Silva (B.6.6.2-06/2). They performed the analysis using the percent pre-implantation loss per litter as experimental unit with a non-parametric test for multiple comparison. It generated a significance value of $p < 0.05$ for the 700 mg/kg bw/day. Indeed, the percentage values for preimplantation loss are 11.3±21.7; 13.4±20.3; 17.4±22.8 and 13.4±11.0*. RAC notes that, otherwise the statistical significance, dose-response is not observed and the atypical small standard deviation for the 700 mg/kg bw/day group is causing a statistical artefact. It is also noted that HCD from the conducting laboratory are unavailable for further evaluating the biological significance of this finding. Overall, RAC concludes that this finding is not of toxicological relevance.

Developmental toxicity study in rabbits (B.6.6.2/03)

In the range finding developmental toxicity study in rabbits (B.6.6.2/03), OPP was administered via gavage at doses of 0, 250, 500 and 750 mg/kg bw/day to pregnant rabbits. Administration of OPP at 750 mg/kg bw/day led to a high mortality rate (5 of 7). One animal at 750 mg/kg bw/day survived to scheduled sacrifice but exhibited clinical signs of "*blood in the pan*" (presumptive abortion); the uterus contained two resorptions.

Clinical signs, such as perineal soiling were observed in all treatment groups. Deaths also occurred in all treatment groups, following a dose-related trend. At ≥ 500 mg/kg bw/day body weight reduction and marked body weight gain depression were shown. At 500 mg/kg bw/day, one surviving rabbit aborted two foetuses on GD20 before sacrifice. At necropsy, absolute and relative kidney weights in animals treated with 500 mg/kg bw/day were significantly increased. Moreover, kidney histopathology, consistent with focal inflammation and tubule degeneration, was seen in most animals; in addition, some animals had gastric mucosa erosion. The administration of 250 mg/kg bw/day also caused decreases in body weight and body weight gain for the duration of the dosing period. A few cases displayed alterations in kidney, such as inflammation and tubule degeneration and one showed autolysis in the liver.

There were increased incidences of litters having resorptions: 43% (3/7), 83% (5/6) and 60% (3/5) at 0, 250, and 500 mg/kg bw/day, respectively. RAC notes that no dose-response was observed in these resorptions and these results were obtained with a relatively low number of animals since this is a range-finding study. The report did not provide data for foetal examinations.

Developmental toxicity study in rabbits (B.6.6.2/04)

The main developmental study with rabbits (B.6.6.2/04) is summarised in the table below. In this study, OPP was administered at doses of 0, 25, 100 and 250 mg/kg bw/day. OPP had no effect on maternal body weight or body weight gain in animals dosed up to 250 mg/kg bw/day. The highest dose of 250 mg/kg bw/day was however toxic to rabbits, four rabbits were found dead, showing ulceration and haemorrhage in the gastric mucosa. Among the clinical signs, does presented reduced activity and faeces content, perineal soiling and faeces stained with blood. The body weight was reduced in this group, but more noticeable was the body weight gain reduction. At necropsy, evidence of maternal toxicity at 250 mg/kg bw/day included renal tubular degeneration and inflammation. Histological examination showed no renal lesions occurred at 0, 25, or 100 mg/kg bw/day but at 250 mg/kg bw/day there was renal tubular degeneration (33% [8/24 litters] incidence).

As the predominant developmental effect, a slight foetal weight reduction was also observed in this 250 mg/kg bw/day group. OPP exerted no significant effect on foetal body weight or litter size nor did it induce external, soft tissue, or skeletal anomalies or malformations (data not shown). The only developmental effect of OPP in rabbits was increased incidence of litters with resorptions (33.3, 57.1, 76.9 and 77.2%, respectively). These percentages exceed the HCD (mean 36.2%, range 11.1-66.7%). The authors of this study dismiss this effect claiming that it was not statistically significant; do not observe dose-response; the high background suggest these animals had a generally higher typical frequency resorptions; and the records are only marginally above the historical controls.

An alternative interpretation of these study's data has been proposed by Kwok and Silva (B.6.6.2-06). The study was conducted in two 2 phases because insufficient pregnant females were available for examination in the high dose group, and in this case additional control and high dose animals were treated approximately one month after the first phase. For reporting purposes, the results of both phases were combined. Kwok and Silva re-evaluated the data using percent resorptions per litter and non-parametric test for dose-response comparison. Using this methodology, they identified statistically significant increases in the frequency of resorptions at 100 and 250 mg/kg bw/day ($p < 0.05$) and dose-response trend (31%, 57%, 77% and 82% for control, 25, 100 and 250 mg/kg bw/day dose groups) when the first phase data were analysed in isolation; while the trend was not significant for the combined data. Kwok and Silva also suggested that two of the non-pregnant females could have been wrongly recorded while they were indeed pregnant but suffered 100% resorption. RAC notes that this statement is unsubstantiated and very hard to admit in an experienced operator, at least without questioning then the validity of the whole study.

Developmental toxicity study in mice (B.6.6.2-05)

The developmental toxicity study in mice (B.6.6.2-05) is summarised in the table below. Four groups of vaginal plugs bearing mice (21 animals/dose) were treated by gavage at 0 (olive oil), 1450, 1740, and 2100 mg/kg bw/day OPP on GD 7 through 15 and sacrificed on GD 18. Dose selection was based on LD_{50} data for OPP in rat (but not mice).

Maternal body weight gain was presented as a graph (no summarised or individual data presented) but it was evident that at the mid- and high dose there was a decrease from the first day of treatment (no statistical analysis provided). A dose-related increase in maternal deaths was observed at all levels with 16/20 dying at the highest dose tested. Although maternal deaths occurred at each dose level, inhibition of maternal body weight gain occurred only at 1740 and 2100 mg/kg bw/day. Therefore, the evidence for maternal toxicity at 1450 mg/kg bw/day (low dose) was 4/21 maternal deaths.

OPP reduced foetal body weight and increased skeletal developmental delays in each of the OPP treated groups, with both changes showing dose dependency. Increased overall incidence of severe external malformations (cleft palate, open eye, and exencephalia) occurred at the low and mid doses. At the high dose, despite having only five litters for examination at laparohysterectomy, the overall incidence of malformations was increased, and when maternal uterine contents were examined, there was a 2.2-fold increased incidence in late foetal resorptions.

Overall, RAC notes that all malformations found in mice were presented at doses largely exceeding 10% of mortality and therefore such malformations cannot substantiate an eventual classification for developmental toxicity.

***Table**: Summary of animal studies on adverse effects on development with OPP. Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr).*

Method	Results	Reference
Developmental Toxicity No guideline Female Wistar rats 11 to 20 /dose OPP purity = 99.7% Oral gavage 0, 150, 300, 600, 1200 mg/kg bw/day from day 6 to 15	**MATERNAL TOXICITY** Mortality: 10/11 dams of the highest dose group died after 3-9 days of treatment Clinical signs: After treatment with ≥ 300 mg/kg bw/day, pregnant rats fell into ataxia for several hours the severity of which was dose-dependent. 600 mg/kg bw/day: ↓ body weight gain [(GD 9 (60%), GD 12 (51%), GD 15 (62%) and GD 20 (46%)] 300 mg/kg bw/day: ↓ body weight gain [(GD 9 (17%), GD 12 (18%), GD 15 (28%) and GD 20 (20%)] **DEVELOPMENTAL TOXICITY** <u>600 mg/kg bw/day</u> • ↑ percentage of foetal death (85%) • ↓ mean foetal weight in ♂/♀ (6%/8%) • ↑ foetal incidence of malformations: o Cranial or sacral meningocele (1/237, 0.4%, n.s.) o Hydronephrosis (14/119, 11.8%, n.s.) o Diaphragmatic hernia) (1/119, 0.8%, n.s.) o Omphalocele (1/188, 0.8%, n.s.) <u>300 mg/kg bw/day</u> • ↑ foetal incidence of malformations: o Cranial or sacral meningocele (2/188, 1.7%) n.s.) o Hydronephrosis (7/97, 7.2%, n.s.) o Diaphragmatic hernia (2/97, 2.1%, n.s.) Maternal LOAEL: 300 mg/kg bw/day Maternal NOAEL: 150 mg/kg bw/day Critical effect at the LOAEL: ↓ bw gain and overt toxicity (ataxia) Developmental LOAEL: 300 mg/kg bw/day Developmental NOAEL: 150 mg/kg bw/day Critical effect at the LOAEL: based on ↑ incidence of foetal malformations (i.e. cranial or sacral meningocele, hydronephrosis, and diaphragmatic hernia).	Kaneda *et al.*, 1978 B.6.6.2/01

<table>
<tr><th>Method</th><th>Results</th><th>Reference</th></tr>
<tr><td>Developmental Toxicity

No guideline

SD female rats

25 to 35/dose

OPP purity = 99.69%

Oral gavage

0, 100, 300, 700 mg/kg bw/day, from day 6 to 15 (inclusive)</td><td>MATERNAL TOXICITY

<u>Mortality:</u> 1/25 (vs. 0/35 in controls) dams died due to an accident during administration of the test substance.

700 mg/kg bw/day:
• ↓ body weight [day 10 (6%) and day 16 (6%)]
• ↓ body weight gain [days 6 to 9 (64%)]
• ↓ absolute liver weight [days 21(18%)]

DEVELOPMENTAL TOXICITY

<u>700 mg/kg bw/day:</u>
• ↑Incidence of post-implantation loss:
o Foetuses: 13.4%
o Litters: 15/20 75%
• Skeletal alteration:
↑Incidence foetuses with:
o Delayed ossification of sternebrae [10/252 (4%) foetuses (f) or 6/20 (30%) litter (l) vs. 5/416 (1%) f or 5/34 (15%)]
o Skull foramen [6/252 (2%) (f) or 6/20 (30%) (l) vs. 5/416 (1%) (f) or 5/34 (15%)]
o Skull bone island [7/252 (3%) (f) or 6/20 (30%) (l) vs. 5/416 (1%) (f) or 4/34 (12%)]

Maternal LOAEL: 700 mg/kg bw/ day

Maternal NOAEL: 300 mg/kg bw/ day

Critical effect at the LOAEL: ↓ body weight gain and ↓ liver weight

Developmental LOAEL: 700 mg/kg bw/ day

Developmental NOAEL: 300 mg/kg bw/ day

Critical effect at the LOAEL: ↑ incidence of skeletal variants and post-implantation loss.</td><td>B.6.6.2/02, 1978</td></tr>
<tr><td>Developmental toxicity

7 NZW female rabbit/dose

OPP (purity 99.77%)

Oral gavage: 0, 250, 500 and 750 mg/ kg bw/day from day 7 to 19 of gestation</td><td>MATERNAL TOXICITY

<u>Mortality:</u> Nine rabbits died prior to study termination. Two rabbits (one at 500 and one at 750 mg/kg bw/day) were found with depositions of the test material in the lungs. The remaining deaths were considered treatment-related.

<u>Clinical signs:</u>

<table>
<tr><td></td><td colspan="4">Dosage (mg/kg bw/day)</td></tr>
<tr><td></td><td>0</td><td>250</td><td>500</td><td>750</td></tr>
<tr><td>Aborted</td><td>0</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>Blood in pan</td><td>0</td><td>0</td><td>1</td><td>2</td></tr>
<tr><td>Blood stained faeces</td><td>0</td><td>1</td><td>0</td><td>0</td></tr>
<tr><td>Faeces-decreased amount</td><td>5</td><td>6</td><td>5</td><td>4</td></tr>
<tr><td>Faeces-soft</td><td>0</td><td>1</td><td>2</td><td>0</td></tr>
<tr><td>Perineal soiling</td><td>0</td><td>1</td><td>2</td><td>2</td></tr>
<tr><td>Abnormal respiration</td><td>0</td><td>0</td><td>0</td><td>2</td></tr>
<tr><td>Thin</td><td>0</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Unsteady in cage</td><td>0</td><td>1</td><td>0</td><td>0</td></tr>
</table>

<u>750 mg/kg bw/day</u>
• ↓ body weight [(GD 13 (20%)]
• ↓ body weight gain [(GD 7-10 (302%) and GD 10-13 (1216%)]</td><td>B.6.6.2/03, 1991b</td></tr>
</table>

Method	Results	Reference
	• Gross pathology: Digestive tract haemorrhage, gaseous distension and erosions of the stomach, decreased/soft ingest of the gastrointestinal tract, haemolysed blood in intestines, pale kidneys. • Histopathology:	

	Dosage (mg/kg bw/day)			
	0	250	500	750
No. examined	7	7	7	7
Kidney				
Autolysis	0	1	2	5
Degeneration tubule(s), bilateral, focal, slight	0	2	3	0
Degeneration tubule(s), bilateral, multifocal, moderate	0	0	1	0
Degeneration tubule(s), bilateral, diffuse, moderate	0	0	0	1
Inflammation, bilateral, focal, slight	0	0	0	1
Inflammation, bilateral, diffuse, moderate	0	2	4	0
Liver				
Autolysis	0	1	2	5
Stomach				
Erosion (s), mucosa, focal, slight	0	0	0	3
Pigment-haematogenous increased, mucosa	0	0	2	3

<u>500 mg/kg bw/day</u>
- ↓ body weight gain [GD 7-10 (101%)]
- ↑ kidney absolute/relative weight (15%, ns/34%)
- Gross pathology: Pale kidneys

<u>250 mg/kg bw/day</u>
- ↑ kidney relative weight (16%, ns)

REPRODUCTIVE PARAMETERS

No statistically significant differences in: number bred, % pregnant, number of deaths, number moribund, pregnancies detected by stain, number of litters totally resorbed, number of viable litters, number of corpora lutea/dam, number of implantations/dam, % preimplantation loss, foetuses/litter, number of resorptions/litter, % implantations resorbed, % litter with resorptions, resorptions/litters with resorptions.

Maternal LOAEL: 250 mg/kg bw/ day

Maternal NOAEL: < 250 mg/kg bw/ day

Critical effect at the LOAEL: ↑ mortality and alterations in the kidneys

A developmental NOAEL cannot be stablished, since foetuses were not examined for skeletal, visceral and external anomalies.

Developmental toxicity 16 to 24 NZW female rabbits/dose OPP (purity 99.77%)	**MATERNAL TOXICITY** Mortality • One control ♀ died on day 16 due to umbilical herniation and volvulus of the jejunum. Another control ♀ was terminated on day 24 after spontaneous abortion.	B.6.6.2/04, 1991c

Method	Results	Reference
Oral gavage: 0, 25, 100, 250 mg/ kg bw/day from day 7 to 19 of gestation	• 2 ♀ (25 mg/kg bw/day) died on day 23: one due to partial blockage of the stomach and intestinal tract due to a large hairball, another one was terminated after spontaneous abortion, occlusion of stomach and intestinal tract due to large hairball and possibility of pregnancy toxaemia. • One ♀ (100 mg/kg bw/day) died on day 14 after inadvertent deposition of the test material into the lungs caused by gavage error. • Five ♀ (250 mg/kg bw/day) died: 4 of them on days 15 and 16 due to treatment-related effects within the gastrointestinal tract (ulceration and haemorrhage of the gastric mucosa, haemolysed blood within the intestinal tract and decreased content and increased fluidity of ingest). Another ♀ was terminated on day 21 after spontaneous abortion (ulceration and haemorrhage of gastric mucosa, plus evidence suggesting renal toxicity were found). Clinical signs	

	Dosage (mg/kg bw/day)			
	0	25	100	250
Number of animals on test	18	16	16	24
Appeared normal	4	1	1	1
Aborted	1	1	0	1
Blood discharge (vulva)	0	1	0	0
Blood in pan	0	1	3	4
Blood stained faeces	0	0	0	3
Broken toenail	0	0	1	0
Cold to the touch	0	1	0	0
Decreased activity	1	1	0	4
Facial soiling - clear	0	0	0	1
Faeces-decreased amount	13	15	12	23
Faeces - soft, loose	3	8	6	8
Found dead	1	1	1	3
Laboured breathing	1	0	0	0
Moist wound on neck - small	0	0	1	0
Moribund	0	0	0	1
No hindleg movements	0	0	0	1
Perineal soiling	3	8	6	10
Urine discoloration - red	1	0	0	2

250 mg/kg bw/day
- ↓ body weight [(GD 0 (3%, ndr)].
- Gross necropsy: Ulceration and haemorrhage of the gastric mucosa, haemolysed blood within intestinal tract and decreased content and increased fluidity of ingest.
- Histopathology of the kidney:

	Dosage (mg/kg bw/day)			
	0	25	100	250
Kidneys (no. of tissues examined)	18	16	16	24
Degeneration, tubule(s), unilateral, focal: - slight	0	0	0	1
Degeneration, tubule(s), bilateral, focal: - slight	0	0	0	2
Degeneration, tubule(s), bilateral, multifocal: - slight	0	0	0	2
Degeneration, tubule(s), bilateral, multifocal: - moderate	0 0	0 0	0 0	3 1
Inflammation, unilateral, focal: - slight	0	0	0	3
Inflammation, bilateral, focal: - slight	0	0	0	4
Inflammation, bilateral,	0	0	0	1

Method	Results	Reference

multifocal: - slight Inflammation, pelvis, unilateral, focal: - slight Inflammation, pelvis, bilateral, focal: - slight	0	0	0	2

REPRODUCTIVE AND LITTER PARAMETERS

250 mg/kg bw/day
- ↑ % litters with resorptions (116%; n.s.; ndr)
- ↑ number of resorptions/litters (22%; n.s.; ndr)
- ↑ post implantation loss (50%; n.s.; ncdr)

100 mg/kg bw/day
- ↑ % litters with resorptions (131%; n.s.; ndr)
- ↑ number of resorptions/litters (55%; n.s.; ndr)
- ↑ post implantation loss (57%; n.s.; ncdr)

25 mg/kg bw/day
- ↑ % litters with resorptions (71%; n.s.; ndr)
- ↑ post implantation loss (37%; n.s.; ncdr)

LITTER PARAMETERS

No statistically significant differences

Maternal LOAEL: 250 mg/kg bw/ day

Maternal NOAEL: 100 mg/kg bw/ day

Critical effect at the LOAEL: ↓ bw gains, ↑ mortality and renal tubular degeneration.

Developmental LOAEL: > 250 mg/kg bw/ day

Developmental NOAEL: ≥ 250 mg/kg bw/ day

Developmental toxicity

No guideline

20 to 21 JCL-ICR female mice /dose

Oral gavage

0, 1450, 1740 and 2100 mg/kg bw/day from day 7 to 15 of gestation

MATERNAL TOXICITY

2100 mg/kg bw/day
- ↑ mortality (76% of unscheduled deaths): 5 mice died on day 8 of gestation, 7 on day 9 and 2 each on days 11 and 12.
- ↓ body weight/body weight gain (no numerical data available)
- ↓ in absolute/relative heart weight (12%/12%)

1740 mg/kg bw/day
- ↑ mortality (33% of unscheduled deaths): 4 mice died on day 7 and 1 each on days 14, 15 and 16 of gestation
- ↓ body weight/body weight gain (no numerical data available)
- ↓ in absolute/relative heart weight (9%/7%) and ↑ in relative liver weight (10%, ndr)

1450 mg/kg bw/day
- ↑ mortality (19% of unscheduled deaths): 2 mice died on days 11 and 15 of gestation and 2 mice died on day
- ↑ in absolute/relative liver weight (15%, ndr/17%, ndr)

LITTER/REPRODUCTIVE DATA

2100 mg/kg bw/day
- ↓ foetal bw in ♂/♀ (20%/20%)
- ↑ frequency of foetuses with cervical ribs (17% vs. 0% in controls)

Ogata *et al.,* 1978

B.6.6.2-05

Method	Results	Reference

- ↓ mean number of ossified left/right phalanges in forelegs (62%/62%) and hindlegs (44%/44%) and posterior lumbar vertebrae (21%)

1740 mg/kg bw/day

- ↓ early resorptions (89%)
- ↓ foetal bw in ♂/♀ (5%/4%)
- ↑ frequency of foetuses with cervical ribs (9% vs. 0% in controls)
- ↓ mean number of ossified left/right phalanges in forelegs (5%/5%)
- ↑ frequency of foetuses with externally visible malformations (6% vs. 0.67% in controls)

1450 mg/kg bw/day

- ↓ early resorptions (53%)
- ↓ foetal bw in ♂/♀ (4%/8%)
- ↑ frequency of foetuses with cervical ribs (7% vs. 0% in controls)
- ↓ mean number of ossified left/right phalanges in hindlegs (7%/5%)
- ↑ frequency of foetuses with externally visible malformations (6% vs. 0.67% in controls)

External malformations

	OPP (mg/kg bw/day)			
	0	1450	1740	2100
External malformations[a]				
No. litters examined	20	14	14	5
Cleft palate	1 [1] 5%	1 [1] 7%	4[4] 29%	1[1] 20%
Open eyelids	1 [1] 5%	4 [7] 29%	6 [6] 43%	1 [1] 20%
Exencephalia	0	3 [6] 21%	0	0
Frequency of foetuses with externally visible malformations (All types combined)[b]	0.67±2.05	6.21±8.03* ↑826%	6.14±5.96* ↑816%	3.64±4.98

a) Number of affected litters, with number of affected foetuses in brackets and the percent of litters affected, as reported by the investigators.
b) * $p<0.05$

Maternal LOAEL: 1450 mg/kg bw/day

Maternal NOAEL: < 1450 mg/kg bw/day

Critical effect at the LOAEL: ↑ mortality.

Developmental LOAEL: 1450 mg/kg bw/day.

Developmental NOAEL: < 1450 mg/kg bw/day.

Critical effect at the LOAEL: ↓ foetal bw, ↑ resorptions, ↑ incidence of skeletal variants and ↑ incidence of foetuses with externally visible malformations.

Comparison with the criteria

Fertility and sexual function

Overall, reproductive parameters were seemingly not affected in rats in two reliable 2-generation reproductive toxicity studies. No human information is available on the effects of OPP on the reproductive system. Consequently, conditions for classification have not been met and classification is not warranted. **RAC supports the DS's proposal for no classification of OPP for fertility and sexual function.**

Development

Only minor delayed ossification in foetuses were found in two developmental toxicity studies in rats, but these effects appeared concurrently with a reduction in maternal body weight gain between gestation days 6-9 of 64% and of 6% in maternal body weight by day 16. One study in rabbits reported increases in percentage of litters with resorptions, number of resorptions with litters and increases in post implantation losses. However, it is noted that these increases were not statistically significant, do not observed dose-response and moreover were not noted in a second study with rabbits at comparable doses.

The meta-analysis performed by Kwok and Silva (B.6.6.2-06) reassessed the developmental and reproductive toxicity studies with OPP. This study raises the possibility that OPP is a developmental toxicant and need to be classified as such. The overall conclusion of this meta-analysis is that there could be a pattern of developmental effects associated with OPP treatment across all species examined. Although further studies are needed to elucidate the developmental toxicity of OPP, this re-evaluation indicated that foetal effects (e.g., resorption) occurred in the absence of maternal toxicity. The relevant maternal and developmental NOAELs in rats treated with OPP were established at 150 mg/kg bw/day, whereas in rabbits the relevant maternal and developmental NOAEL after OPP treatment were proposed to be 100 mg/kg bw/day and 250 mg/kg bw/day, respectively. In the meeting (Peer review of the pesticide risk assessment of the active substance 2-phenylphenol, EFSA Scientific Report, 2008; 217, 1-67) it was considered that the developmental NOAEL should be lowered from 250 mg/kg bw/day to 100 mg/kg bw/day based on some foetus resorptions in rabbits. However, there was not a clear teratogenic response and the meeting concluded that the NOAEL of 250 mg/kg bw/day was appropriate.

RAC notes that a dose-related and statistically significant increase of litter resorption in the B.6.6.2/04 study is revealed when data only from the first testing phase of the study is used. The reasons of Kwok and Silva for not considering the second testing phase of the study is that only two control does and six does at 250 mg/kg bw/day were investigated. It poses a concern about the possibility that OPP is a teratogen. However, this possibility is not definitively proven since the reasons for not considering the results of the second phase are not well substantiated (the authors of the meta-analysis considered a misclassification between non-pregnant females and pregnant females with 100% resorptions) and, whether there are reasons for questioning the results of one phase, these same reasons could be also automatically considered for another phase.

In an expert position paper submitted during the consultation, the PPP notifier claimed the evidence suggests that the statistical significance in resorptions detected by Kwok and Silva could not be of biological relevance because no maternal toxicity was reported at 100 mg/kg bw/day. Consequently, RAC notes that, if not caused by maternal toxicity or stress, the foetal resorption was due to OPP toxicity. In this case, less extreme forms of toxicity could have been expected in parallel. However, no effects on foetal body weights or on visceral or skeletal abnormalities were observed. Overall, RAC considers the result of the B.6.6.2/04 study as inconclusive since the doubt whether the statistical differences in resorptions reported by Kwok and Silva is artefactual or of toxicological relevance remains.

In the two 2-generation studies, both conducted in albino Sprague-Dawley rats, the main teratogenic effect noted in pups was observed in kidney at high doses tested in presence of maternal toxicity. In the first generational study (B.6.6.1-01), renal pelvis dilation was found in pups (21 days and older), however, this effect cannot be attributed to OPP administration by the following reasons:

- The incidence was increased only in a dose-related manner in F1a females, but not in F1b/F2a/F2b females or males
- Not present in both generations, which would be indicative of a treatment-related effect
- HCD from reproduction studies using albino Crl:CD(SD)BR rats showed that dilated renal pelvis in weanling and cull control animals was common

In summary, RAC notes certain concern with the results of the developmental toxicity study in rabbits, but at the same time, with the available information, the reported effects are not enough to warrant classification and consequently the Committee **supports the DS's proposal for no classification of OPP for developmental toxicity.**

Effects on or via lactation

There were no indications of impaired nursing behaviour or decreased pup viability during lactation in the 2-generation reproductive toxicity study. This study does not provide indications that OPP could alter quality of the breast milk. There is no toxicokinetic indications that allow assume that OPP is being transferred to breast milk at significant levels. Overall, **RAC supports the DS's proposal for no classification of OPP for effects on or via lactation.**

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

DS proposed no classification of OPP for aspiration toxicity

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Comparison with the criteria

RAC proposes no classification of OPP for aspiration toxicity due to lack of data.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

OPP is listed in Annex VI of the CLP Regulation with Aquatic Acute 1 classification. The Dossier Submitter (DS) proposed to classify the substance as Aquatic Acute 1, M-factor of 1 and Aquatic Chronic 1, M-factor of 1.

Some of the aquatic toxicity studies have been performed with sodium 2-biphenylate. Under environmentally relevant pH conditions sodium 2-biphenylate (SOPP) will dissociate on contact with water forming hydrolysed Na+ and OH- ions and the protonated OPP (OPP). Consequently, dissociation of sodium 2-biphenylate to OPP is also relevant for toxicity testing. Testing of sodium 2-biphenylate for effects in the environment will include the formation of OPP and a differentiation between the effect of the molecules is not feasible. OPP and SOPP are expected to have a similar environmental fate and ecotoxicity profile due to the comparable chemical structures and physicochemical properties of both substances.

Degradation

Biodegradation of OPP (OPP) was 100% after 14 days in a Modified OECD Screening Test (OECD TG 301E) (Kanne 1989a). In another OECD TG 301E test using Rhine River water as inoculum, OPP degraded 100% after 6 days (Kanne 1989b). The DS considered the substance as readily biodegradable.

OPP was hydrolytically stable in an OECD TG 111 Hydrolysis test at pH 4, pH 7 and pH 9 (Reusche 1990).

OPP was rapidly photodegraded (experimental DT_{50} 0.3 days) in sterile aqueous 0.01 M phosphate buffer. Based on the experimental DT_{50} the predicted DT_{50} was calculated to be 1.7 solar summer days at Phoenix, USA or 2.6 summer days at Athens, Greece (Heinemann 2002). The DS concluded that OPP is not likely to be photolytically stable in aqueous medium. The major metabolite was diketohydroxy-compound with DT_{50} of 1.3 days, equivalent to 7.2 solar summer days in Phoenix, USA and 11.1 summer days in Athens, Greece.

There was no OECD TG 308 water/sediment study or a surface water simulation study available.

The DS considered OPP to be **rapidly degradable**.

Bioaccumulation

There was a bioconcentration test for fish available performed according to EC C.13 (1998) which is equivalent to OECD TG 305. Test substance concentrations were 5.0 and 50 µg OPP (OPP)/L. Fish were exposed to test substance for an uptake phase of 53 hours. Concentrations of OPP were determined by HPLC in test waters and fish samples at intervals throughout the study. The lipid content of the fish was determined at the start of the uptake phase and end of the depuration phase. The steady state bioconcentration factor (BCF) was determined as 21.7. The BCF values with consideration of the lipid content of fish were 114 and 115 at 5 µg OPP/L and 50 µg OPP/L, respectively. The DS concluded that OPP had a **low potential for bioaccumulation**.

Also, the Log K_{OW} of 3.18 at 22.5 ºC (pH6.3) (OECD TG 117) also indicated a low potential for bioaccumulation.

Aquatic toxicity

Acute aquatic toxicity

Table *Summary of reliable information on acute aquatic toxicity*

Method	Test material (*	Species	Results	Reference
Fish				
ASTM Standard E729-80 (similar to OECD TG 203), GLP Static	99.25% OPP	*Pimephales promelas* *Pimephales promelas* *Lepomis macrochirus* *Oncorhynchus mykiss*	96-h LC_{50} = 4.7 mg/L 96-h LC_{50} = 5.5 mg/L 96-h LC_{50} = 4.6 mg/L 96-h LC_{50} = 4.0 mg/L nominal, measured 98-105% of nom.	B.9.2.1/01 (1985)
UBA method 1984 (similar to OECD TG 203), GLP Semi-static	99.5% OPP	*Danio rerio*	96-h LC_{50} = 4.5 mg/L (nom) measured ≥80% of nom.	B.9.2.1/02 (1989a)
OPPTS Draft 850.1075 (similar to OECD TG 203), GLP Flow-through	71.48% SOPP (98.16% SOPP tetrahydrate)	*Oncorhynchus mykiss*	96-h LC_{50} = 2.6 mg SOPP/L (mm) measured 67-83% of nom.	B.9.2.1/04 (2006a)
OPPTS Draft 850.1075 (similar to OECD TG 203), GLP Flow-through	71.48% SOPP (98.16% SOPP tetrahydrate)	*Cyprinodon variegatus*	96-h LC_{50} = 5.1 mg SOPP/L (mm) measured 52-80% of nom.	B.9.2.1/05 (2006b)
OPPTS Draft 850.1075 (similar to OECD TG 203), GLP Flow-through	71.48% SOPP (98.16% SOPP tetrahydrate)	*Lepomis macrochirus*	96-h LC_{50} = 5.1 mg SOPP/L (mm) measured 61-80% of nom.	B.9.2.1/06 (2006c)
Invertebrates				
ASTM Standard E729-80 (similar to OECD 202), GLP Static	99.25% OPP	*Daphnia magna*	48-h EC_{50} = 2.7 mg/L nominal, measured >80% of nom.	B.9.2.4.1/01 (1985)
OPPTS Draft 850.1035, GLP Flow-through	**71.48% SOPP (98.16% SOPP tetrahydrate)**	***Americamysis bahia***	**96-h LC_{50} = 0.32 mg SOPP/L (mm) measured 55-80% of nom.**	**B.9.2.4.2/01 (2006d)**
OPPTS Draft 850.1025, GLP Flow-through	71.48% SOPP (98.16% SOPP tetrahydrate)	*Crassostrea virginica*	96-h EC_{50} = 3.4 mg SOPP /L (mm) Shell growth measured 80-120% of nom.	B.9.2.4.2/02 (2006)

Method	Test material (*	Species	Results	Reference
Algae and aquatic plants				
OECD TG 201, GLP static	99.91%	*Selenastrum capricornutum*	72-h E_rC_{50} = 3.57 mg/L (mm) measured 82-88% of nom.	B.9.2.6.1/01 (2002)
OPPTS draft 850.4400 (similar to OECD TG 221), GLP semi-static	71.48% SOPP (98.16% SOPP tetrahydrate)	*Lemna gibba*	7-day EC_{50}: 6.2 mg SOPP/L (frond density >9.4 mg SOPP/L (growth rate) 7.7 mg SOPP/L (frond biomass) (mm) measured 77-94% of nom.	B.9.2.7/01 (2006)

(* In SOPP studies the test substance was OPP/SOPP, Dowicide® A Antimicrobial (sodium salt orthophenyl phenol) (draft RAR Volume 3 – B.9 (AS) May 2021)

There were reliable acute aquatic toxicity OPP (OPP) data available for fish, *Daphnia magna* and algae. In addition, there are data available for sodium 2-biphenylate (SOPP) on fish, *Americamysis bahia, Crassostrea virginica* and *Lemna gibba*. The lowest toxicity value was a 96-hour LC_{50} of 0.32 mg SOPP/L for *Americamysis bahia*.

The acute toxicity of sodium 2-biphenylate to *Americamysis bahia* was determined in a flow-through test design using artificial seawater as test medium. The mysids were exposed for 96 hours to nominal substance concentrations of 0.13, 0.22, 0.36, 0.60, and 1.0 mg a.i./L. The test solutions were replaced at a rate of 90% every 6 hours. The test substance concentration was analytically verified at test initiation and test termination by HPLC. The mean measured concentrations were 0.071, 0.16, 0.25, 0.44, and 0.80 mg a.i./L (55, 71, 71, 73, and 80% of nominal). Mortality, abnormal behaviour or appearance of the test organism were recorded at 24, 48, 72 and 96 hours. Dissolved oxygen ranged from 7.3 to 8.6 mg/L, pH from 8.1 to 8.3 and temperature from 19 to 25°C. Following 96 hours of exposure, 5, 10, 20, 75, and 100% mortality was observed among mysids exposed to the 0.071, 0.16, 0.25, 0.44, and 0.80 mg/L treatment levels, respectively. Although mortality of 5% was observed in the lowest treatment level tested (0.071 mg/L), this is considered to be within the expected range of variability for acute tests and not toxicant-related. No mortality or sublethal effects were observed among mysids exposed to the control.

Chronic aquatic toxicity

Table: *Summary of reliable information on chronic aquatic toxicity*

Method	Test material (*	Species	Results	Reference
Fish				
Harries et al 2000 (** (similar to OECD TG 234,229 and 230), GLP Flow-through	99.9% OPP	*Pimephales promelas*	21-day NOEC = 0.036 mg/L (mm) fecundity, hatchability measured 59-81% of nom.	B.9.2.2.2/01 (2002)

Method	Test material (*	Species	Results	Reference
Invertebrates				
OECD TG 211, GLP Semi-static	99.85% OPP	*Daphnia magna*	21-day NOEC 0.006 mg/L (mm) reproduction nominal 0.01, 0.03 and 0.1 mg OPP/L → geometric mean 0.006, 0.011 and 0.024 mg/L	B.9.2.5.1/01 (2001)
OECD TG 219, GLP Static, sediment-water study	100% OPP	*Chironomus riparius*	28-d EC_{50} = 3.35 mg/L 28-d NOEC 1.85 mg/L (mm) (***	B9.2.5.3/01 (2005)
Algae and aquatic plants				
OECD 201, US-EPA OPPTS 850.5400, GLP Vehicle acetone	99.91% OPP	*Selenastrum capricornutum*	72-h NOEC = 0.468 mg/L (mm) measured 82-88% of nom.	B.9.2.6.1/01 (2002)
OPPTS draft 850.4400 (similar to OECD TG 221), GLP semi-static	71.48% SOPP (98.16% SOPP tetrahydrate)	Lemna gibba	7-day NOEC: 2.3 mg SOPP/L (frond density, growth rate, frond biomass) (mm) measured 77-94% of nom.	B.9.2.7/01

(*In SOPP studies the test substance was OPP/SOPP, Dowicide® A Antimicrobial (sodium salt orthophenyl phenol) (draft RAR Volume 3 – B.9 (AS) May 2021)

(** Harries *et al.*, 2000, Development of a reproductive performance test for endocrine disrupting chemicals using pair-breeding fathead minnows (*Pimephales promelas*). Environmental Science and Technology, 34, 3003-3011.

(***OPP rapidly dissipates from water to sediment, then dissipates from the sediment at a slower rate

For chronic toxicity there were OPP (OPP) data available for fish, *Daphnia magna, Chironomus riparius*, and algae. A *Lemna gibba* study was available for sodium 2-biphenylate (SOPP). The lowest chronic toxicity value was a 21-day NOEC (reproduction) of 0.006 mg OPP/L for *Daphnia magna*.

The influence of OPP on survival, reproductive capacity and behaviour of *Daphnia magna* was tested over 21 days under semi-static exposure conditions. The test was undertaken according to OECD TG 211 and following GLP. Young female Daphnia were exposed to the test substance at nominal concentrations of 0.01, 0.03, and 0.1 mg/L. The living offspring was counted three times a week, along with the renewal of the test media. Concentrations were analysed during the study. Arithmetic mean measured concentrations were included in the laboratory report. The results were accepted but measured concentrations were recalculated on the basis of geometric means due to differences among the measured concentrations and some values below the LOQ. The geometric mean measured concentrations were 0.006, 0.011, and 0.024 mg a.i./L. These values were used for estimation of the endpoints.

In conclusion, the Dossier Submitter (DS) proposed to retain the short-term classification and add an M-factor of 1 ($0.1 < LC_{50} \leq 1$ mg/L) and also proposed adding a long-term classification of Aquatic Chronic 1, M=1. The substance is rapidly degradable and has a low potential for bioaccumulation. The lowest acute toxicity value was a 96-hour EC_{50} of 0.32 mg sodium 2-biphenylate/L for *Americamysis bahia* warranting Aquatic Acute 1 classification with an M-factor

of 1 (0.1 < EC_{50} ≤ 1). The lowest chronic toxicity value was a NOEC of 0.006 mg OPP/L for *Daphnia magna* warranting Aquatic Chronic 1 classification with an M-factor of 1 for a rapidly degradable substance (0.001 mg/L < NOEC ≤ 0.01 mg/L).

Comments received during consultation

Comments were received from one Member State (MS). They agreed to the proposed classification. They wanted more information on the relation between monitoring data and rapid degradation. The DS clarified that the substance is shown to be rapidly degradable according to the CLP Criteria based on testing. The monitoring data presented in the CLH Report are mainly focused on other purposes and as a widely used substance observed contamination in municipal STP discharges is not surprising. RAC agrees that there is no need to consider monitoring data to assess rapid degradability in the case where data preferred according to the criteria is available. Overall, monitoring data is very difficult to use for classification purposes as described in the CLP Guidance (II.2.3.3).

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS to consider OPP as rapidly degradable based on:

- 100% degradation in an OECD TG 301E Ready biodegradability test after 14 days
- 100% degradation in an OECD TG 301E Ready biodegradability test after 6 days
- the substance was hydrolytically stable in an OECD TG 111 Hydrolysis test at pH 4, pH 7 and pH 9

Bioaccumulation

RAC agrees with the DS to consider OPP as being non-bioaccumulative based on the fish BCF values of 114 and 115 which are below the cut-off value of 500 in the CLP Criteria. This is supported by the Log K_{OW} of 3.18 being below the cut-off criteria of 4.

Aquatic toxicity

RAC agrees with the DS proposal to base the short-term aquatic classification on the 96-hour LC_{50} of 0.32 mg SOPP/L for *Americamysis bahia*. Under environmentally relevant pH conditions sodium 2-biphenylate (SOPP) will dissociate on contact with water forming hydrolysed Na+ and OH- ions and the protonated 2-phenylphenol (OPP). Testing of sodium 2-biphenylate for effects in the environment will include the formation of 2-phenylphenol and a differentiation between the effect of the molecules is not feasible. OPP and SOPP are expected to have a similar environmental fate and ecotoxicity profile due to the comparable physicochemical properties of both substances.

Consequently, RAC agrees with the DS that OPP warrants classification as Aquatic Acute 1 classification with an M-factor of 1 (0.1 mg/L < LC50 ≤ 1 mg/L).

Chronic toxicity

RAC agrees with the DS proposal to base the long-term aquatic classification on the NOEC of 0.006 mg OPP/L for *Daphnia magna* which warrants Aquatic Chronic 1, M=1 classification for a rapidly degradable substance (0.001 mg/L < NOEC ≤ 0.01 mg/L).

Consequently, RAC agrees with the DS that OPP warrants classification as **Aquatic Acute 1, H400, M=1 and Aquatic Chronic 1, H410, M=1.**

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

The estimated DT_{50} of OPP (OPP) in air is 0.59 days (tropospheric) (APOWIN v.1.91). Considering the chemical structure and other available information on the physicochemical properties, OPP is not expected to be hazardous to stratospheric ozone. Despite the small amount of information available, the DS proposed no classification due to insufficient data to reach a conclusive outcome..

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC agrees with the DS conclusion that there is no available evidence concerning the properties and predicted or observed environmental fate and behaviour of OPP to indicate that it may be present a danger to the structure and/or functioning of the stratospheric ozone layer. RAC agrees to not classify the substance as hazardous to the ozone layer but believes that there is adequate information to conclude on no classification.

ANNEXES:

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

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