Committee for Risk Assessment

RAC

Annex 1
Background document
to the 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

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

Adopted

2 December 2022

European Commission

Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

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

Volume 1

Rapporteur Member State: Spain
Co-Rapporteur Member State: Greece

November 2021

Version History

When	What
2020/3	Level 3. Criteria – Article 4 and annex II of regulation (EC) No 1107/2009
2020/10	Draft Renewal Assessment Report (dRAR) – prepared in the context of the application for renewal of approval of the a.s. according to Reg (EU) No 844/2012
January 2021	Initial RAR-RMS Spain
May 2021	DRAR after CoRMS & Applicant comments
September 2021	Document amended following ECHA review for CLH proposal
November 2021	DRAR after EFSA CoCh

The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report should include the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS. Nevertheless, the views and conclusions of the RMS should always be clearly and transparently reported; the conclusions from the applicant should be included as an Applicant's statement for every single study reported at study level; and the RMS should justify the final assessment for each endpoint in all cases, indicating in a clear way the Applicant's assessment and the RMS reasons for supporting or not the view of the Applicant.

Table of contents

Level 1

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

1 STATEMENT OF SUBJECT MATTER AND PURPOSE FOR WHICH THIS REPORT HAS BEEN PREPARED AND BACKGROUND INFORMATION ON THE APPLICATION

1.1 CONTEXT IN WHICH THIS DRAFT ASSESSMENT REPORT WAS PREPARED

1.1.1 Purpose for which the draft assessment report was prepared

According to Commission Directive 2009/160/EU of 17 December 2009 amending Council Directive 91/414/EEC to include 2-phenylphenol as active substance:

The Commission Regulations (EC) Nº 1112/2002 and (EC) Nº 2229/2004 lay down the detailed rules for the implementation of the fourth stage of the programme of work referred to in Article 8(2) of Directive 91/414/EEC and establish a list of active substances to be assessed, with a view to their possible inclusion in Annex I to Directive 91/414/EEC. That list includes 2- phenylphenol, and it effects on human health and the environment have been assessed in accordance with the provisions laid down in Regulations (EC) No 1112/2002 and (EC) No 2229/2004 for a range of uses proposed by the notifier. Moreover, those Regulations designate the rapporteur Member States which have to submit the relevant assessment reports and recommendations to the European Food Safety Authority (EFSA) in accordance with Article 22 of Regulation (EC) No 2229/2004. For 2-phenylphenol the rapporteur Member State was Spain and all relevant information was submitted on 11 February 2008.

The assessment report has been peer reviewed by the Member States and the EFSA and presented to the Commission on 19 December 2008 in the format of the EFSA Scientific Report for 2-phenylphenol. This report has been reviewed by the Member States and the Commission within the Standing Committee on the Food Chain and Animal Health and finalised on 27 November 2009 in the format of the Commission review report for 2-phenylphenol.

It has appeared from the various examinations made that plant protection products containing 2-phenylphenol may be expected to satisfy, in general, the requirements laid down in Article 5(1)(a) and (b) of Directive 91/414/EEC, in particular with regard to the uses which were examined and detailed in the Commission review report. Because of this it was appropriate to include 2- phenylphenol in Annex I, in order to ensure that in all Member States the authorisations of plant protection products containing this active substance can be granted in accordance with the provisions of that Directive. Without prejudice to that conclusion, it was appropriate to obtain further information on certain specific points. Article 6(1) of Directive 91/414/EEC provides that the inclusion of a substance in Annex I may be subject to conditions. Therefore it was appropriate to require that the notifier submit further information on the potential for skin depigmentation for workers and consumers due to possible exposure to the metabolite 2-phenylhydroquinone (PHQ) on citrus peel. In addition, the notifier should submit further information to confirm that the analytical method applied in residue trials correctly quantifies the residues of 2-phenylphenol, PHQ and their conjugates.

A reasonable period was allowed to elapse before an active substance was included in Annex I in order to permit Member States and the interested parties to prepare themselves to meet the new requirements which will result from the inclusion.

According to Commission Directive 2010/81/EU of 25 November 2010 amending Council Directive 91/414/EEC as regards an extension of the use of the active substance 2-phenylphenol on 18 June 2010 the notifier submitted information on other application techniques, such as wax treatment, dipping treatment and foam curtain treatment, in order to remove the restriction to closed drench chambers. Spain, which had been designated rapporteur Member State by Commission Regulation (EC) No 2229/2004, evaluated the additional information and submitted to the Commission on 30 July 2010 an addendum to the draft assessment report on 2-phenylphenol, which was circulated for comments to the other Member States and to the European Food Safety Authority (EFSA). In the comments received no major concerns were raised and the other Member States and EFSA did not raise any point which would exclude the extension of the use. The draft assessment report together with that addendum was reviewed by the Member States and the Commission within the Standing Committee on the Food Chain and Animal Health and finalised on 28 October 2010 in the format of the Commission review report for 2-phenylphenol. The new information on the application techniques submitted by the notifier and the new assessment carried out by the rapporteur Member State indicate that plant protection products containing 2-phenylphenol may be expected to satisfy, in general, the requirements laid down in Article 5(1)(a) and (b) of Directive 91/414/EEC, in particular with regard to the indoor uses as a post-harvest fungicide which were examined and detailed in the Commission review report. Consequently, it was no longer necessary to restrict the use of 2-phenylphenol to closed drench chambers, as laid down in Directive 91/414/EEC as amended by

Directive 2009/160/EU. Without prejudice to that conclusion, it was appropriated to obtain further information on certain specific points. Article 6(1) of Directive 91/414/EEC provides that inclusion of a substance in Annex I may be subject to conditions. Therefore, it was appropriate to require that the notifier submit further information to confirm the residue levels occurring as a result of application techniques other than those in drench chambers.

The Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances, shows the date of approval as 1 January 2010 and the expiration of inclusion as 31 December 2019 for 2-phenylphenol.

On 17 May 2013 the Standing Committee on the Food Chain and Animal Health had taken note of the revision of this review report after the assessment of the above confirmatory data. This assessment had been carried out in line with the Guidance document on the procedures for submission and assessment of confirmatory data following inclusion of an active substance in Annex I of Council Directive 91/414/EEC7. The Committee agreed that, on the basis of the current outcome, the analytical method applied in residue trials could be confirmed. The use of 2-phenylphenol as post-harvest fungicide did not arised concerns as regards the potential for skin depigmentation. Therefore, the conclusions of the original risk assessment are not substantially modified by the evaluation of the submitted confirmatory data. No further review by EFSA had been considered necessary.

At Commission Implementing Regulation (EU) 2017/555 of 24 March 2017 amending Implementing Regulation (EU) No 540/2011 as regards the extension of the approval periods of several active substances listed in Part B of the Annex to Implementing Regulation (EU) No 686/2012 (AIR IV renewal programme) in the sixth column, expiration of approval, of row 299, 2-phenylphenol (including its salts such as the sodium salt), the date is replaced by 31 December 2021.

The application is for the renewal of 2-Phenylphenol (incl. sodium salt orthophenyl phenol) and as such is based on representative use patterns reflecting the range of existing and proposed uses for products containing OPP in the EU.

The GAP table below lists the intended uses supported in the EU for which data have been provided in the renewal dossier. The representative formulation is AGF/1-04, an EC formulation containing 100 g/L of OPP and used as a drencher.

Following a pre-submission meeting with RMS Spain on the 1st December 2016, it was agreed that residues data for the two other types of formulation of OPP currently available on the market would be submitted as part of this Annex I Renewal submission. The two other formulations are:

- **AGF/1-03, an SL formulation containing 130 g/L OPP and used as a foam curtain**
- **AGC/1-10, an EW formulation containing 2.5 g/L OPP and used as a wax**

The submission of this extra information is to facilitate the review of Maximum Residue Level values. The GAP tables for these two formulations are also presented below (1.5.1).

Lanxess Deutschland GmbH makes this submission in their capacity as manufacturer of OPP.

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

According to Commission Implementing Regulation (EU) No 686/2012 of 26 July 2012 allocating to Member States, for the purposes of the renewal procedure, the evaluation of the active substances whose approval expires after 31 December 2018 and not later than 31 December 2021 the latest Spain has been designated as the Rapporteur Member State (RMS) and Greece as the Co-rapporteur Member State (Co-RMS).

For the purposes of the renewal procedure, the evaluation of each active substance set out in the first column of the Annex, is allocated to a rapporteur Member State, as set out in the second column of that Annex, and to a co-rapporteur Member State, as set out in the third column of that Annex.

PART B

Allocation of the evaluation of active substances whose approval expires after 31 December 2018 and not later than 31 December 2021

Active substance	Rapporteur Member State	Co-rapporteur Member State

2-Phenylphenol (incl. sodium salt orthophenyl phenol)	ES	EL

Spain as RMS produced a frist version of the DRAR of the active substance 2-Phenylphenol that was distributed for comments to the CoRMS (Greece) and the applicant in January 2021. Comments received from the CoRMS and applicant was taken into consideration for producing the version of the DRAR that was sent to EFSA for the *peer review*, a reporting table was produced will all the received comments.

1.1.3 EU Regulatory history for use in Plant Protection Products

2-Phenylphenol (incl. sodium salt orthophenyl phenol) Dossier Submission Under Commission Regulation (EU) 844/2012

Lanxess Deutschland GmbH hereby submited the dossier according to Commission Regulation (EU) 844/2012 of 18 September 2012 for the renewal of the regulatory approval of 2-Phenylphenol (incl. sodium salt orthophenyl phenol) (OPP) under Commission Regulation (EC) 1107/2009.

OPP was included in Annex I of Council Directive 91/414/EC and is an approved active substance under Regulation (EC) 1107/2009 as specified in Commission Implementing Regulation (EU) 540/2011 of 25 May 2011. The review report for OPP (SANCO/10698/2009 – rev 3, 17 May 2013) provides conclusions and end points agreed in the original EU review for Annex I inclusion.

Commission Regulation (EU) 2017/555, amending implementing Regulation (EU) 540/2011 as regards the extension of the approval periods of certain substances, prolongs the inclusion of OPP until 31/12/2021.

Successful notification for the inclusion of OPP at Annex I was made by Lanxess Deutschland GmbH.

Lanxess Deutschland GmbH own all of the data used in the active substance part of this Annex I renewal submission. The data in the representative product part of the dossier are supplied by Agrupación Española de Servicios y Procesos Postcosecha AIE (AGRUPOST). The relevant letters of access are provided in Document B of the dossier.

1.1.4 Evaluations carried out under other regulatory contexts

This substance has been reviewed for use as a biocide in the EEA under the Biocidal Products Regulation (EU) No 528/2012. However, the submitted CLH-report has been withdrawn on 7 October 2020.

1.2 APPLICANT INFORMATION

1.2.1 Name and address of applicant(s) for approval of the active substance

Company name: Lanxess Deutschland GmbH
Address: Kennedyplatz 1
50569 Köln
Germany

1.2.2 Producer or producers of the active substance

Company name: Lanxess Deutschland GmbH
Address: Kennedyplatz 1
50569 Köln
Germany

1.2.3 Information relating to the collective provision of dossiers

Lanxess Deutschland GmbH are sole supporters of this Active Substance Renewal dossier for 2-phenylphenol (incl. sodium salt orthophenyl phenol) (OPP).

A Task Force was formed with the purpose of supporting OPP through the previous Active Substance Renewal process. Membership of the OPP Task Force were:

LANXESS Deutschland GmbH
D-51369 Leverkusen
Germany

And

DOW Benelux B.V.
Herbert H. Dowweg 5
NL-4530 Terneuzen
The Netherlands

All test reports generated or sponsored either by Lanxess Deutschland GmbH or its affiliates or Dow Benelux B.V. or its affiliates and filed in this submission are co-owned by both parties.

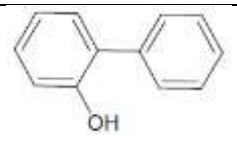
Studies generated or sponsored by Agrupación Española de Servicios y Procesos Postcosecha AIE (AGRUPOST) or Productos Citrosol S.A. are owned by Agrupación Española de Servicios y Procesos Postcosecha AIE (AGRUPOST) or Productos Citrosol S.A; Lanxess Deutschland GmbH has the right to use and cite these studies.

OPP was once owned by Bayer Chemicals. All rights and data were transferred to Lanxess Deutschland GmbH in 2004.

Please refer to the following documents for details of the access rights of Lanxess Deutschland GmbH to the studies:

1. OPP Letter of Access – Lanxess Deutschland GmbH
2. OPP Letter of Access – Dow Benelux B.V.
3. OPP Letter of Access – Productos Citrosol S.A
4. OPP Letter of Access – Agrupost
5. Bayer Chemicals to Lanxess Deutschland GmbH
6. Bayer AG to Bayer Chemicals AG.

1.3 IDENTITY OF THE ACTIVE SUBSTANCE

1.3.1 Common name proposed or ISO-accepted and synonyms	2-phenylphenol (ISO) Synonyms: biphenyl-2-ol (EINECS name), OPP
1.3.2 Chemical name (IUPAC and CA nomenclature)	
IUPAC	2-phenylphenol, *o*-phenylphenol
CA	[1,1'-Biphenyl]-2-ol
1.3.3 Producer's development code number	Not applicable
1.3.4 CAS, EEC and CIPAC numbers	
CAS	90-43-7
EEC	201-993-5
CIPAC	246
1.3.5 Molecular and structural formula, molecular mass	
Molecular formula	$C_{12}H_{10}O$
Structural formula	
Molecular mass	170.2 g/mol
1.3.6 Method of manufacture (synthesis pathway) of the active substance	CONFIDENTIAL information - data provided separately (Volume 4)
1.3.7 Specification of purity of the active substance in g/kg	998 g/kg minimum.
1.3.8 Identity and content of additives (such as stabilisers) and impurities	
1.3.8.1 Additives	CONFIDENTIAL information - data provided separately (Volume 4)
1.3.8.2 Significant impurities	CONFIDENTIAL information - data provided separately (Volume 4)
1.3.8.3 Relevant impurities	None.
1.3.9 Analytical profile of batches	CONFIDENTIAL information - data provided separately (Volume 4)

1.4 INFORMATION ON THE PLANT PROTECTION PRODUCT

<table>
<tr><td>1.4.1 Applicant</td><td>Lanxess Deutschland GmbH</td></tr>
<tr><td>1.4.2 Producer of the plant protection product</td><td>Lanxess Deutschland GmbH</td></tr>
<tr><td>1.4.3 Trade name or proposed trade name and producer's development code number of the plant protection product</td><td>Code number: AGF/1-04</td></tr>
<tr><td colspan="2">1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product</td></tr>
<tr><td>1.4.4.1 Composition of the plant protection product</td><td>Pure active substance
<table>
<tr><td>Content of pure active substance[1] :</td><td>100 g/L</td><td>(9.49 % w / w)*</td></tr>
<tr><td>limits :
(±10%)</td><td>(90– 110)
g / L</td><td>(8.54 – 10.44)
% w / w</td></tr>
</table>
Technical active substance
<table>
<tr><td>Content of technical active substance[1] :</td><td>100.2 g / L</td><td>(9.51 % w / w)*</td></tr>
<tr><td>limits :
(±10%)</td><td>(90.2 – 110.2)
g / L</td><td>(8.56 – 10.46)
% w / w</td></tr>
</table>
*based on a density of 1054 g/L.
[1] At a minimum purity of the technical active substance of 99.8 %.</td></tr>
<tr><td>1.4.4.2 Information on the active substances</td><td>
<table>
<tr><th>Type</th><th>2-Phenylphenol</th></tr>
<tr><td>ISO common name</td><td>2-phenylphenol</td></tr>
<tr><td>CAS No</td><td>90-43-7</td></tr>
<tr><td>EC No</td><td>201-993-5</td></tr>
<tr><td>CIPAC No</td><td>246</td></tr>
<tr><td>Salt, ester anion or cation present</td><td>Anionic form may be present when in water solution equilibrium (pka=9.5).</td></tr>
</table>
</td></tr>
<tr><td>1.4.4.3 Information on safeners, synergists and co-formulants</td><td>CONFIDENTIAL information – data provided separately (Vol 4)</td></tr>
<tr><td>1.4.5 Type and code of the plant protection product</td><td>Emulsifiable Concentrate [Code : EC]</td></tr>
<tr><td>1.4.6 Function</td><td>Fungicide</td></tr>
</table>

1.4.7 Field of use envisaged	OPP is used as a post-harvest fungicide for control of fungi in citrus fruits. It is currently applied to citrus fruits as a wax, a foam or a drench. OPP also has biocidal applications, such as hygienic handwashes, hard-surface liquid disinfectants, livestock housing disinfectants, industrial/institutional premises disinfectants, in-can preservatives and metal-working fluid preservatives.
1.4.8 Effects on harmful organisms	OPP is a broad spectrum, contact fungicide used to prevent the growth of fungi on citrus fruits during storage.

1.5 DETAILED USES OF THE PLANT PROTECTION PRODUCT

1.5.1 Details of representative uses

RMS: Information from document D1 is adapted to the GAP according the latest agreed template.

Crop and/or situation (a)	**Member State**	**Product Name**	**F G I** (b)	**Pests or group of pests controlled** (c)	**Formulation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc of a.i. g/kg (i)	Method kind (f-h)	Growth stage and season (j)	Number min max (k)	Interval between applications (min)	Kg a.i./hl min max (g/hl) (l)	Water l/ha min max	Kg a.i./ha min max (*) (g/ha) (l)		
Citrus fruits	Spain	AGF/1-04	I	Post-harvest fungi	EC	100 g/L	Drencher	Post harvest	a) 1 b) 1	n/a	0.05-0.06	n/a	n/a	n/a	Application rate = 0.5 – 0.6 L product/hL (50-60 g a.s./hL)

n/a: not applicable

* For uses where the column "Remarks" in marked in grey further consideration is necessary. Uses should be crossed out when the notifier no longer supports this use(s).

(a) For crops, the EU and Codex classification (both) should be taken into account ; where relevant, the use situation should be described (e.g. fumigation of a structure)

(b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)

(c) e.g. biting and suckling insects, soil born insects, foliar fungi, weeds

(d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)

(e) GCPF Codes – GIFAP Technical Monograph N° 2, 1989

(f) All abbreviations used must be explained

(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench

(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant – type of equipment used must be indicated

(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). In certain cases, where only one variant synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).

(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application

(k) Indicate the minimum and maximum number of application possible under practical conditions of use

(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha

(m) PHI - minimum pre-harvest interval

Summary of additional intended uses that in addition to the uses above, have also been considered in the consumer risk assessment (*2-Phenylphenol (incl. sodium salt orthophenyl phenol)*). Residues data for the two other types of formulation of OPP currently available on the market would be submitted as part of this Annex I Renewal submission. The two other formulations are:

- **AGF/1-03, an SL formulation containing 130 g/L OPP and used as a foam curtain**
- **AGC/1-10, an EW formulation containing 2.5 g/L OPP and used as a wax**

The submission of this extra information is to facilitate the review of Maximum Residue Level values. The GAP tables for these two formulations are also presented below.

Important note: efficacy, environmental risk and risk to humans by exposure other than via their diet have not been assessed for these uses

Crop and/or situation (a)	**Member State**	**Product Name**	**F G I** (b)	**Pests or group of pests controlled** (c)	**Formulation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc of a.i. g/kg (i)	Method kind (f-h)	Growth stage and season (j)	Number min max (k)	Interval between applications (min)	Kg a.i./hl min max (g/hl) (l)	Water l/ha min max	Kg a.i./ha min max (*) (g/ha) (l)		
Citrus fruits	Spain	AGF/1-03	I	Post-harvest fungi	SL	130 g/L	Foam curtain	Post harvest	1	n/a	n/a	n/a	n/a	n/a	Application rate = 0.2 L product/tonne
Citrus fruits	Spain	AGC/1-10	I	Post-harvest fungi	EW	2.5 g/L	Wax	Post harvest	1	n/a	n/a	N/A	n/a	n/a	Application rate = 1 L product/tonne

n/a: not applicable

* For uses where the column "Remarks" in marked in grey further consideration is necessary. Uses should be crossed out when the notifier no longer supports this use(s).

(a) For crops, the EU and Codex classification (both) should be taken into account ; where relevant, the use situation should be described (e.g. fumigation of a structure)

(b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)

(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds

(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)

(e) GCPF Codes – GIFAP Technical Monograph N° 2, 1989

(f) All abbreviations used must be explained

(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench

(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant – type of equipment used must be indicated

(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). **In certain cases, where only one variant synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).**

(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application

(k) Indicate the minimum and maximum number of application possible under practical conditions of use

(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha

(m) PHI - minimum pre-harvest interval

1.5.2 Further information on representative uses

Application Rate and Concentration of Active Substance
The proposed application rate of AGF/1-04 is 0.5 to 0.6 L product/hL, which is equivalent to 50-60 g OPP/hL of water.
The proposed application rate of AGF/1-03 is 0.2 L product/tonne of fruit, which is equivalent to 26 g OPP/tonne of fruit.
The proposed application rate of AGC/1-10 is 1.0 L product/tonne of fruit, which is equivalent to 2.5 g OPP/tonne of fruit.

Method of Application
AGF/1-04 is applied as a drencher.
AGF/1-03 is applied as a foam curtain.
AGC/1-10 is applied as a wax.

Number and Timings of Applications and Duration of Protection
AGF/1-04, AGF/1-03 and AGC/1-10 are applied once, post-harvest, to citrus fruits. The application of these products is a preventative measure to stop fruit spoilage by fungi. All three formulations are efficacious for the amount of time that the fruit are in storage.

Necessary Waiting Periods or Other Precautions to Avoid Phytotoxic Effects on Succeeding Crops
AGF/1-04, AGF/1-03 and AGC/1-10 are applied post-harvest, therefore there is no preharvest interval. There is no waiting time before workers can re-enter the crop.
AGF/1-04, AGF/1-03 and AGC/1-10 are applied post-harvest, therefore there are no effects on succeeding crops.

Proposed Instructions for Use
The label of AGF/1-04 is presented in document C of this dossier. For convenience, the instructions for use from the label of AGF/1-04 are:
Dilute the product at a concentration of 0.5 – 0.6L product per 100L water.
Application to fruit is by means of a drencher system for 25-30 seconds.
The fruit must be allowed to drain and dry well.
Treated fruit in the EU must be labelled “fruits treated with orthophenylphenol fungicide” in accordance with Regulation 543/2011 laying down detailed rules for the application of Council Regulation (EC) No 1234/2007 in respect of the fruit and vegetables and processed fruit and vegetables sectors.

If the treated fruit are exported then the national legislation must be followed.

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

Summary of additional intended uses that in addition to the uses above, have also been considered in the consumer risk assessment (OPP) Regulation (EC) N° 1107/2009 Article 8.1(g))

Important note: efficacy, environmental risk and risk to humans by exposure other than via their diet have not been assessed for these uses

Crop and/or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (m)	Remarks
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
MRL Application (according to Article 8.1(g) of Regulation (EC) No 1107/2009)															
Citrus fruits	Spain	AGF/1-03	I	Post-harvest fungi	SL	130 g/L	Foam curtain	Post-harvest	1	n/a	n/a	n/a	n/a	n/a	Application rate = 0.2L product/tonne fruit
Citrus fruits	Spain	AGC/1-10	I	Post-harvest fungi	EW	2.5 g/L	Wax	Post-harvest	1	n/a	n/a	n/a	n/a	n/a	Application rate = 1.0L product/tonne fruit

(a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)
(c) *e.g.* biting and sucking insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalogue of pesticide
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indicated
(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). **In certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).**
(j) Growth stage range from first to last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of applications possible under practical conditions of use
(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha
(m) PHI - minimum pre-harvest interval

1.5.4 Overview on authorisations in EU Member States

OPP is used as a post-harvest fungicide in citrus. It was first suggested for this purpose in the 1930s. It is currently applied to citrus fruits as a wax, a foam or a drench. Post-harvest products containing OPP that are currently marketed by various companies in Europe are shown in the table below.

Table 1.5.4. List of Currently Authorised Uses and Extent of Use

Country	Product Name	Product Details	Registration No.	Registration Holder	Current Crop Uses
Spain	Britex-F	0.65% OPP + 18% waxes	13094	CIA. Iberica Brogdex S.A.	Post-harvest citrus
Spain	Briozil	10% OPP + 7.5% imazalil	22868	CIA. Iberica Brogdex S.A.	Post-harvest citrus
Spain	Citrashine N-PE	0.25% OPP	16233	Decco Iberica Post Cosecha S.A.U.	Post-harvest citrus
Cyprus	Citrocil	10% OPP + 7.5% imazalil	3049	Productos Citrosol S.A.	Post-harvest citrus
Croatia	Citrocil	10% OPP + 7.5% imazalil	UP/I-320-20/14-01/479	Productos Citrosol S.A.	Post-harvest citrus
Portugal	Citrocil	10% OPP + 7.5% imazalil	0259	Productos Citrosol S.A.	Post-harvest citrus
Spain	Citrocil	10% OPP + 7.5% imazalil	18537	Productos Citrosol S.A.	Post-harvest citrus
Spain	Citrosol A OPP	0.25% OPP	ES-00171	Productos Citrosol S.A.	Post-harvest citrus
Spain	Decco-OPP	10% OPP	24751	Decco Iberica Post Cosecha S.A.U.	Post-harvest citrus
Spain	Deccosol-MF	13% OPP	11312	Decco Iberica Post Cosecha S.A.U.	Post-harvest citrus
Spain	Foamer	13% OPP	15608	Fomesa Fruitech S.L.	Post-harvest citrus
Spain	Foamex	13% OPP	15041	CIA. Iberica Brogdex S.A.	Post-harvest citrus
Spain	Fruitgard-OPP	10% OPP	25355	Fomesa Fruitech S.L.	Post-harvest citrus
Spain	Fung-cid Orto Espuma	13% OPP	22600	Productos Citrosol S.A.	Post-harvest citrus
Spain	Ortocil	10% OPP	24783	Productos Citrosol S.A.	Post-harvest citrus
Spain	Ortodex	28.6% Na-OPP	23602	CIA. Iberica Brogdex S.A.	Post-harvest citrus
Spain	Ortosol 6500	28.6% Na-OPP	23374	Productos Citrosol S.A.	Post-harvest citrus
Spain	Textar 10 OP	10% OPP	25635	Tecnidex S.A.	Post-harvest citrus
Spain	Textar 13 OP	13% OPP	21086	Tecnidex S.A.	Post-harvest citrus
Spain	Teycer C OP	0.25% OPP + 18% waxes	21087	Tecnidex S.A.	Post-harvest citrus
Spain	Teycer DB-OP	13% OPP	16092	Tecnidex S.A.	Post-harvest citrus
Spain	Waterwax 2P	0.25% OPP	15650	Fomesa Fruitech S.L.	Post-harvest citrus

Level 2

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

2 SUMMARY OF ACTIVE SUBSTANCE HAZARD AND OF PRODUCT RISK ASSESSMENT

Summary of methodology proposed by the applicant for literature review and for all sections

The literature search report is summarised below.

Databases used in literature search

The following databases were used for the literature search:

- PubMed
- MEDLINE

PubMed provides free access to MEDLINE. MEDLINE includes citations regarding a range of subjects including biology, environmental science, marine biology, plant and animal science as well as biophysics and chemistry. The majority of citations are from scholarly journals.

Timeframe of literature search

The timeframe of publication of references in the literature search was 01/2009 to 01/2019.

2.1 IDENTITY

2.1.1 Summary or identity

2-Phenylphenol (incl. sodium salt orthophenyl phenol), OPP (ISO common name: o-phenylphenol) has a minimum purity of 998 g/kg. There are no manufacturing impurities considered to be of toxicological concern.

2.2 PHYSICAL AND CHEMICAL PROPERTIES [EQUIVALENT TO SECTION 7 OF THE CLH REPORT TEMPLATE]

2.2.1 Summary of physical and chemical properties of the active substance

Table 1: Summary of physicochemical properties of the active substance

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Technical material: Solid colourless flakes, slight phenolic odour. Purified material: Colourless solid, slight phenolic odour.	KCA, 2.2/01 Stroech, K. 2006 B.2.3/01	Estimated
Melting/freezing point	56.7 °C	KCA, 2.1/01 Erstling K., 2001 and 2006, Study no. A 00/0068/01/LEV B.2.1/01	Measured
Boiling point	287 °C	KCA, 2.1/02 Erstling K., 2001 and 2006, Study no. A 00/0068/01/LEV B.2.1/02	Measured
Relative density	D_4^{20} = 1.237	KCA, 2.1/01 Erstling K.,	Measured

Property	Value	Reference	Comment (e.g. measured or estimated)
		2001 and 2006, Study no. A 00/0068/01/LEV B.2.14/03	
Vapour pressure	The measurements have been carried out in the temperature range from 1°C up to approximately 30°C and a regression calculation has been performed: OPP Vapour pressure: 0.474 Pa at 20°C 0.906 Pa at 25°C 16.2 Pa at 50°C (extrapolated)	KCA, 2.2/01 Olf, 2003, Study no. 03/003/01 B.2.2/01	Measured
Surface tension	58.72 mN/m at 20.1°C (90% saturated solution in pure water, 0.558 g/L) OPP is surface active.	KCA 2.12/01 Olf G., 2004 Study no. 04/006/03 B.2.12/01	Measured
Water solubility	**pH 5**: 0.43 g/L at 10°C; 0.53 g/L at 20°C; 0.70 g/L at 30°C **pH 7**: 0.45 g/L at 10°C; 0.56 g/L at 20°C; 0.73 g/L at 30°C **pH 9:** 0.52 g/L at 10°C; 0.64 g/L at 20°C; 0.84 g/L at 30°C	KCA, 2.5/01 Erstling, 2002 A Study no. 00/0068/02/LEV B.2.5/01	Measured
Partition coefficient n-octanol/water	Log Pow (pH 6.3) = 3.18 at 22.51°C Although the substance is surface active the highest concentration of the test substance in water is only 0.6 mg/L and therefore the effect of surface activity is negligible. Both phases were separated in a separatory funnel and centrifuged. Clear solutions were obtained.	KCA, 2.7/01 Kausler, 1991 Study no. A 89/0062/06/LEV Feldhues, 2007a (amendment No. 2 to A 89/0062/06/LEV) Feldhues, 2007b (statement partition coefficient n-octanol/water of Preventol O extra pH dependence) Study no. A 89/0062/06/LEV) B.2.7/01	Measured
Henry's law constant	0.15 $Pa \cdot m^3 \cdot mol^{-1}$ at pH5 (20ºC) 0.14 $Pa \cdot m^3 \cdot mol^{-1}$ at pH7 (20ºC) 0.13 $Pa \cdot m^3 \cdot mol^{-1}$ at pH9 (20ºC)	KCA, 2.2/02 B.2.2/02	Calculated
Flash point	Not required as the melting point is more than 40°C.	KCA, 2.1/01 Erstling K., 2001 and 2006, Study no. A 00/0068/01/LEV B.2.10/01	Estimated

Property	Value	Reference	Comment (e.g. measured or estimated)
	When a flame is applied, OPP melts without ignition.	KCA, 2.9/01 Heinz,U. 2004 Study no. 04/00223 B.2.10/01	Measured
Flammability	OPP is not highly flammable. It does not liberate gases in hazardous amounts when contact with water and does not deliver indications of pyrophoric properties during the realisation of tests according to EC A.10 and EC A.12.	KCA, 2.9/01 Heinz,U. 2004 Study no. 04/00223 B.2.9/01 B.2.14/01 B.2.14/02	Measured
Explosive properties	Based on scientific judgement it is certified that due to the structural formula, OPP contains neither oxidising groups nor other chemically unstable functional groups. Thus, OPP is incapable of rapid decomposition with evolution of gases or release of heat, i.e. the solid material does not present any risk for explosion.	KCA, 2.11/01 Stroech, 2004b B.2.11/01	Estimated
Self-ignition temperature	OPP does not undergo spontaneous combustion heating up to 420°C. No exothermic effects were detected after 27 hours at 140°C.	KCA, 2.9/01 Heinz,U. 2004 Study no. 04/00223 KCA, 2.9/02 Krack M., 2018 Report no. PS20180102-1 B.2.9/02	Measured
Oxidising properties	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. Therefore, OPP does not have oxidising properties.	KCA 2.13/01 Stroech, K. 2004c B.2.13/01	Estimated
Granulometry	Not applicable.		
Solubility in organic solvents and identity of relevant degradation products	<u>OPP Solubility at 20°C:</u> n-heptane 50.3 g/L p-xylene 590 g/L 1,2-dichloromethane 791 g/L methanol 982 g/L acetone 958 g/L ethyl acetate 867 g/L	KCA, 2.6/01 Jungheim, 2004 Study no. A 02/0162/04 B.2.6/01	Measured
Dissociation constant	pKa value = 9.4 ± 00.15 at 20°C Based on scientific chemical judgement, it is certified that due to the structural formula OPP dissociates in an equilibrium reaction, like any other organic phenol, into its associate phenate ion and a proton. The reaction is fully reversible.	KCA, 2.8/01 Kausler, 1991 Study no. A 89/0062/06/LEV KCA, 2.8/02 Feldhues, 2007 (amendment No. 2 to A 89/0062/06/LEV) KCA, 2.8/03 Stroech, 2004a B.2.8/01	Measured (Titration)

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td>Viscosity</td><td>Not applicable.</td><td></td><td></td></tr>
<tr><td>Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity</td><td>UV-Vis spectrum (in acetonitrile):
<table><tr><th>λmax [nm]</th><th>ε [Lmol^{-1} cm^{-1}]</th></tr><tr><td>245</td><td>12800</td></tr><tr><td>287</td><td>8200</td></tr></table>IR spectra:
Structure confirmed using FTIR KBr cell.

<b>^{3}H-NMR and ^{13}C-NMR spectra:
Structure confirmed using acetone-d6 solvent.

MS spectra:
Structure confirmed using electron impact ionisation.</td><td>KCA, 2.4/01
KCA, 2.4/02
KCA, 2.4/03
KCA, 2.4/04

Erstling, K. 2004 A
Study no.
02/0162/03/LEV

B. 2.4/01
B 2.4/02
B. 2.4/03
B. 2.4/04</td><td>Measured</td></tr>
</table>

2.2.1.1 Evaluation of physical hazards [equivalent to section 8 of the CLH report template]

2.2.1.1.1 Explosives *[equivalent to section 8.1 of the CLH report template]*

Table 2: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
-	Non-explosive	Estimated	KCA, 2.11/01 Stroech, 2004b B.2.11/01

2.2.1.1.1.1 Short summary and overall relevance of the provided information on explosive properties

No experimental data are available to evaluate the explosive properties of OPP. Based on scientific judgement it is certified that due to the structural formula, OPP contains neither oxidising groups nor other chemically unstable functional groups. Thus, OPP is incapable of rapid decomposition with evolution of gases or release of heat, i.e. the solid material does not present any risk for explosion.

2.2.1.1.1.2 Comparison with the CLP criteria

OPP does not contain any chemical groups associated with explosive properties as specified in Tables 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.

2.2.1.1.1.3 Conclusion on classification and labelling for explosive properties

Not classified – conclusive but not sufficient for classification.

2.2.1.1.2 Flammable gases (including chemically unstable gases) *[equivalent to section 8.2 of the CLH report template]*

Table 3: Summary table of studies on flammable gases (including chemically unstable gases)

Method	Results	Remarks	Reference
Hazard class not applicable (solid)			

2.2.1.1.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Hazard class not applicable (solid).

2.2.1.1.2.2 Comparison with the CLP criteria

Hazard class not applicable (solid).

2.2.1.1.2.3 Conclusion on classification and labelling for flammable gases

Hazard class not applicable (solid).

2.2.1.1.3 Oxidising gases *[equivalent to section 8.3 of the CLH report template]*

Table 4: Summary table of studies on oxidising gases

Method	Results	Remarks	Reference
Hazard class not applicable (solid)			

2.2.1.1.3.1 Short summary and overall relevance of the provided information on oxidising gases

Hazard class not applicable (solid).

2.2.1.1.3.2 Comparison with the CLP criteria

Hazard class not applicable (solid).

2.2.1.1.3.3 Conclusion on classification and labelling for oxidising gases

Hazard class not applicable (solid).

2.2.1.1.4 Gases under pressure *[equivalent to section 8.4 of the CLH report template]*

Table 5: Summary table of studies on gases under pressure

Method	Results	Remarks	Reference
Hazard class not applicable (solid)			

2.2.1.1.4.1 Short summary and overall relevance of the provided information on gases under pressure

Hazard class not applicable (solid).

2.2.1.1.4.2 Comparison with the CLP criteria

Hazard class not applicable (solid).

2.2.1.1.4.3 Conclusion on classification and labelling for gases under pressure

Hazard class not applicable (solid).

2.2.1.1.5 Flammable liquids *[equivalent to section 8.5 of the CLH report template]*

Table 6: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
Hazard class not applicable (solid)			

2.2.1.1.5.1 Short summary and overall relevance of the provided information on flammable liquids

Hazard class not applicable (solid).

2.2.1.1.5.2 Comparison with the CLP criteria

Hazard class not applicable (solid).

2.2.1.1.5.3 Conclusion on classification and labelling for flammable liquids

Hazard class not applicable (solid).

2.2.1.1.6 Flammable solids *[equivalent to section 8.6 of the CLH report template]*

Table 7: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EC A.10 GLP: Yes	OPP is not highly flammable. Purity: 99.87 % (Sample No. 13947/2002; Batch No. CHHYD P0071)		KCA, 2.9/01 Heinz, U. 2004 Study no. 04/00223 B.2.9/01

2.2.1.1.6.1 Short summary and overall relevance of the provided information on flammable solids

In an A.10 study, 2-phenylphenol did not ignite on contact with the ignition source.

2.2.1.1.6.2 Comparison with the CLP criteria

2-Phenylphenol did not ignite on contact with the ignition source according to the method EC A.10, therefore, the criteria for classification as a flammable solid are not met.

2.2.1.1.6.3 Conclusion on classification and labelling for flammable solids

Not classified – conclusive but not sufficient for classification.

2.2.1.1.7 Self-reactive substances *[equivalent to section 8.7 of the CLH report template]*

Table 8: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
No data provided			

2.2.1.1.7.1 Short summary and overall relevance of the provided information on self-reactive substances

No data are available to evaluate this hazard.

2.2.1.1.7.2 Comparison with the CLP criteria

A self-reactive substance corresponds to a thermally unstable solid liable to undergo a strongly exothermic decomposition even without participation of oxygen (air).

2-Phenylphenol 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.

2.2.1.1.7.3 Conclusion on classification and labelling for self-reactive substances

Not classified – conclusive but not sufficient for classification.

2.2.1.1.8 Pyrophoric liquids *[equivalent to section 8.8 of the CLH report template]*

Table 9: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
Hazard class not applicable (solid)			

2.2.1.1.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Hazard class not applicable (solid).

2.2.1.1.8.2 Comparison with the CLP criteria

Hazard class not applicable (solid).

2.2.1.1.8.3 Conclusion on classification and labelling for pyrophoric liquids

Hazard class not applicable (solid).

2.2.1.1.9 Pyrophoric solids *[equivalent to section 8.9 of the CLH report template]*

Table 10: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
Statement	OPP does not deliver indications of pyrophoric properties during the realization of other tests as defined in EC-A.10 and EC-A.12.	Estimated	KCA, 2.9/01 Heinz, U. 2004 Study no. 04/00223 B.2.9/01

2.2.1.1.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

No data have been provided using test N.2 in Part III, sub-section 33.3.1.4 of the UN RTDG, Manual of Tests and Criteria. 2-Phenylphenol does not deliver indications of pyrophoric properties during the realization of tests as defined in EC-A.10 and EC-A.12. Furthermore, 2-phenylphenol does not ignite spontaneously in contact with air based on experience of handling and use.

2.2.1.1.9.2 Comparison with the CLP criteria

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 2-phenylphenol spontaneously igniting when in contact with air. Therefore, 2-phenylphenol does not meet the criteria for classification as a pyrophoric solid.

2.2.1.1.9.3 Conclusion on classification and labelling for pyrophoric solids

Not classified – conclusive but not sufficient for classification.

2.2.1.1.10 Self-heating substances *[equivalent to section 8.10 of the CLH report template]*

Table 11: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
UN Test N.4 for self-heating substances 100 mm sample cube at 140 ºC GLP: Yes	Negative Preventol O Extra Purity: 99.9 %; Batch No. CHHYDU0242		KCA, 2.9/02 Krack, M. (2018) Report no. PS20180102-1 B.2.9/02

2.2.1.1.10.1 Short summary and overall relevance of the provided information on self-heating substances

2-Phenylphenol was tested for self-heating properties under the test method UN Test N.4 for self-heating substances. Under the conditions of the study, no exothermic effects were detected after 27 hours at 140°C. OPP is not self-heating.

2.2.1.1.10.2 Comparison with the CLP criteria

A negative result has been obtained with 2-phenolpheno in the UN Test N.4 for self-heating substances. Therefore, the criteira for classification of this hazard class has not been met.

2.2.1.1.10.3 Conclusion on classification and labelling for self-heating substances

Not classified – conclusive but not sufficient for classification.

2.2.1.1.11 Substances which in contact with water emit flammable gases *[equivalent to section 8.11 of the CLH report template]*

Table 12: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
EC-A.12 GLP: Yes	OPP does not liberate gases in hazardous amounts		KCA 2.9/01 Heinz, U. (2004) B.2.9/01

2.2.1.1.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

2-Phenylphenol was tested according to the EC-A.12 method. OPP does not liberate gases in hazardous amounts when in contact with water.

2.2.1.1.11.2 Comparison with the CLP criteria

The method EC-A.12 is not suitable for classification purposes of this hazard property according to CLP. However, according to Section 2.12.4.1 of Annex I of CLP, 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 experience in production or handling shows that the substance does not react with water or 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 2-phenylphenol.

2.2.1.1.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Not classified – conclusive but not sufficient for classification.

2.2.1.1.12 Oxidising liquids *[equivalent to section 8.12 of the CLH report template]*

Table 13: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
Hazard class not applicable (solid)			

2.2.1.1.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Hazard class not applicable (solid).

2.2.1.1.12.2 Comparison with the CLP criteria

Hazard class not applicable (solid).

2.2.1.1.12.3 Conclusion on classification and labelling for oxidising liquids

Hazard class not applicable (solid).

2.2.1.1.13 Oxidising solids *[equivalent to section 8.13 of the CLH report template]*

Table 14: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
Statement	Non-oxidising		KCA 2.13/01 Stroech, K. 2004c B.2.13/01

2.2.1.1.13.1 Short summary and overall relevance of the provided information on oxidising solids

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. Therefore, OPP does not have oxidising properties.

2.2.1.1.13.2 Comparison with the CLP criteria

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 of mixture contains oxygen and this element is chemically bound only to carbon or hydrogen. 2-Phenylphenol 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.

2.2.1.1.13.3 Conclusion on classification and labelling for oxidising solids
OPP does not have oxidising properties

Not classified – conclusive but not sufficient for classification.

2.2.1.1.14 Organic peroxides *[equivalent to section 8.14 of the CLH report template]*

Table 15: Summary table of studies on organic peroxides

Method	Results	Remarks	Reference
No data available			

2.2.1.1.14.1 Short summary and overall relevance of the provided information on organic peroxides

2-Phenylphenol is not an organic peroxide. It does not contain the bivalent O-O functional group.

2.2.1.1.14.2 Comparison with the CLP criteria

Hazard class not applicable.

2.2.1.1.14.3 Conclusion on classification and labelling for organic peroxides

Hazard class not applicable.

2.2.1.1.15 Corrosive to metals *[equivalent to section 8.15 of the CLH report template]*

Table 16: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference
No data provided			

2.2.1.1.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No data derived in accordance with the recommended test method in CLP (test in Part III; sub-section 37.4 of the UNRTDG Manual of Tests and Criteria) have been provided.

2.2.1.1.15.2 Comparison with the CLP criteria

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'. 2-Phenylphenol 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 test. Furthermore, evidence from manufacture and handling shows that 2-phenylphenol is not corrosive to metals. Therefore, 2-phenylphenol does not meet the criteria for classification as corrosive to metals.

2.2.1.1.15.3 Conclusion on classification and labelling for corrosive to metals

Not classified – conclusive but not sufficient for classification.

2.2.2 Summary of physical and chemical properties of the plant protection product

AGF/1-04 is an EC formulation containing 10% 2-phenylphenol (OPP). It is a uniform, clear, slight yellowish liquid. It is not explosive and is not flammable. It has a flashpoint of 106°C. The pH of a 1% emulsion of AGF/1-04 in water is 8.74. It has a dynamic viscosity of 409 mPas and a surface tension (in a 1g/L aqueous dilution) of 40.9 mN/m, both at 20°C. The relative density is 1.054. AGF/1-04 is stable after 2 weeks at 54°C, 1 week at 0°C and 2 years at 20°C. In a persistent foam test the foam of a 0.6% aqueous dilution decreased from 20.5ml to 12.9ml after 12 minutes. A 0.6% aqueous dilution of AGF/1-04 re-emulsified fully with no phase separation after 24 hours. The majority of residue was removed in a pourability test (from 2.1% residue before rinsing to 0.2% residue after rinsing).

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).

2.3 DATA ON APPLICATION AND EFFICACY

2.3.1 Summary of effectiveness

OPP is used as a post-harvest treatment for control of fungi in citrus fruits. The key pests include, but are not restricted to:

- *Penicillium digitatum*
- *Penicillium italicum*
- *Phomopsis citri*

OPP shows multi-site activity in fungi. It is adsorbed to the fungal cell membrane, where it disturbs cell membrane functions, such as substrate transport and ATP synthesis. The cell membrane loses its semi-permeability leading to loss of organic molecules and ions.

2.3.2 Summary of information on the development of resistance

OPP is not specifically listed in the Fungicide Resistance Action Committee FRAC Code List of 2018. There is no know OPP resistance in the EU of fungal species causing storage spoilage of citrus fruits.

2.3.3 Summary of adverse effects on treated crops

Adverse effects are not likely to occur in treated crops as the application is a post-harvest treatment on harvested citrus fruits. There is no exposure to citrus trees.

2.3.4 Summary of observations on other undesirable or unintended side-effects

There are no other undesirable or unintended side effects resulting from the use of OPP according to good agricultural practice.

2.4 FURTHER INFORMATION

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

Handling
Avoid formation of respirable particles.
Do not breathe vapours/dust.
Avoid exposure - obtain special instructions before use.
Avoid contact with skin and eyes.
For personal protection see section 8 of the MSDS provided in Document H
Smoking, eating and drinking should be prohibited in the application area.
Provide sufficient air exchange and/or exhaust in work rooms.
Dispose of rinse water in accordance with local and national regulations.
Avoid dust formation. Provide appropriate exhaust ventilation at places where dust is formed.
When using do not eat or drink. When using do not smoke. Wash hands before breaks and at the end of workday.

Storage
Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully re-sealed and kept upright to prevent leakage. Observe label precautions. Electrical installations / working materials must comply with the technological safety standards.

Transport

Transport of dangerous goods	UN number	UN proper shipping name	Transport hazard class	PG	GHS pyctogram	Special precautions
ADR/RID Class	UN3077	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (2-hydroxybiphenyl)	9	III		Special regulations: 274, 335, 375, 601 Tunnel restriction Not applicable Limited quantities 5 kg
IMDG Class	UN3077	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (2-hydroxybiphenyl)	9	III		Special regulations: 274, 335, 966, 967, 969 EmS codes F-A, S-F Limited quantities 5 kg
IATA Class	UN3077	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (2-hydroxybiphenyl)	9	III		Packing instructions: 956 Special provisions: A97, A158, A179, A197(LQ)

PG*: Packing group

RMS: Suggests insertion of standard table above covering all modes of transport and applicable manuals according to section 14 of OPP SDS.

Fire-fighting measures
Suitable extinguishing media: Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.
Unsuitable extinguishing media: High volume water jet
Special protective equipment for firefighters: Wear self-contained breathing apparatus for firefighting if necessary.

Further information: Collect contaminated fire extinguishing water separately. This must not be discharged into drains. Fire residues and contaminated fire extinguishing water must be disposed of in accordance with local regulations.

Special Hazards

Specific hazards during fire-fighting: Do not allow run-off from firefighting to enter drains or water courses.
Hazardous combustion products: Carbon dioxide (CO_2), Carbon monoxide.

2.4.2 Summary of procedures for destruction or decontamination

Containment of spillages

Prevent product from entering drains.
Prevent further leakage or spillage if safe to do so.

Decontamination

If the product contaminates rivers and lakes or drains inform respective authorities.

Disposal

Product: The product should not be allowed to enter drains, water courses or the soil.
Do not contaminate ponds, waterways or ditches with chemical or used container.
Send to a licensed waste management company.

Contaminated packaging: Empty remaining contents.
Dispose of as unused product.
Do not re-use empty containers.

2.4.3 Summary of emergency measures in case of an accident

Protection of emergency workers, bystanders and residents
Emergency workers: Use personal protective equipment.
Avoid dust formation.
Avoid breathing dust.
Bystanders and residents will not be exposed to OPP when used in accordance with the proposed GAP as the application occurs indoors.

First aid measures
General advice:
Move out of dangerous area.
Show this safety data sheet to the doctor in attendance.
Do not leave the victim unattended.

<u>If inhaled:</u>
If unconscious, place in recovery position and seek medical advice.
If symptoms persist, call a physician.
<u>In case of skin contact:</u>
If skin irritation persists, call a physician.
If on skin, rinse well with water.
If on clothes, remove clothes.
<u>In case of eye contact:</u>
Immediately flush eye(s) with plenty of water.
Remove contact lenses.
Protect unharmed eye.
Keep eye wide open while rinsing.
If eye irritation persists, consult a specialist.
<u>If swallowed:</u>
Do NOT induce vomiting.

Keep respiratory tract clear.
Do not give milk or alcoholic beverages.
Never give anything by mouth to an unconscious person.

2.5 METHODS OF ANALYSIS

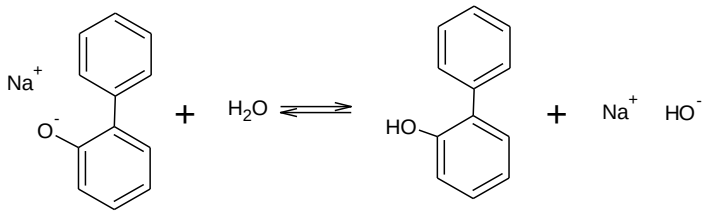
This section summarises the analytical methods for the determination of OPP and its relevant metabolite phenylhydroquinone (PHQ) for the purposes of risk assessment and enforcement.

Sodium orthophenyl phenol (SOPP)

SOPP and its conjugated acid ortho-Phenylphenol (OPP) exist in aqueous solutions in a pH dependant equilibrium.

Under neutral and acidic conditions, the equilibrium shown in Figure 2.8- 2 is shifted to the side of protonated OPP. At high pH values the anionic form is predominant (pKa = 9.5) .

Figure 2.5- 1: Equilibrium of SOPP and OPP (pKa = 9.5) in aqueous solution



Analytical method for the determination of Na-OPP (SOPP) for the purposes of risk assessment (ecotoxicology) is provided in this supplementary dossier. Information about post-approval control and monitoring purposes are not required.

Analytical methods for determination of free OPP can be also used for the determination of SOPP.

2.5.1 Methods used for the generation of pre-authorisation data

2.5.1.1 Analysis of the active substance as manufactured

The analysis of OPP in OPP technical grade active substance is determined by GC-FID.
For further details of the analytical method please refer to confidential Part C.

2.5.1.2 Formulation analysis

The quantification of OPP in a formulated product AGF/1-04 is determined by HPLC-UV.
The test substance is dissolved in acetonitrile, then analysed by HPLC-UV. OPP is identified by retention time through comparison with a reference item.
Analytical methods are also provided for the formulated products AGF/1-03 and AGC/1-10. OPP is determined by HPLC- UV for both formulations.
AGF/1-03 is dissolved in acetonitrile prior to analysis. AGC/1-10 is dissolved in methanol and a buffer solution containing sodium acetate and acetic acid prior to analysis.

2.5.1.3 Methods for Risk Assessment

Plants and plant products

For quantification of OPP in plant matrices, the following methods were developed and validated:

- GC-MS – OPP was extracted from citrus samples by a one-step hydrolysis / steam distillation / extraction procedure and partitioned into isooctane prior to analysis by gas chromatography using mass spectrometer detection. The quantifier ion for OPP was *m/z* 170.

- HPLC-UV – This method was used for validation of the extraction procedure described above. Hydroquinone was quantified at 280 nm.
- HPLC-MS/MS – OPP was extracted from citrus samples with acetonitrile after hydrolysis, with the exception of citrus oil samples, which were extracted with acetone and petroleum ether after hydrolysis. Acetonitrile extracts were diluted in ultra-pure water prior to quantification. Acetone and petroleum ether extracts were evaporated and reconstituted in ultra-pure water prior to quantification. Residues of OPP are quantified using *m/z* 169 to 115 as primary ions and *m/z* 169 to 93 as confirmatory ions (*m/z* 169 to 141 for oil).

For quantification of metabolite PHQ in plant matrices, the following methods were developed and validated:

- GC-MS – PHQ was extracted from citrus samples using dichloromethane following heating at 100°C with ascorbic acid, EDTA and aqueous hydrochloric acid. Extracts are concentrated and cleaned by solid phase extraction prior to analysis by gas chromatography using mass spectrometer detection. The quantifier ion for PHQ was *m/z* 186.
- HPLC-UV – This method was used for validation of the extraction procedure described above. Hydroquinone was quantified at 280 nm.
- HPLC-MS/MS – PHQ was extracted from citrus samples with dichloromethane after hydrolysis, with the exception of citrus oil samples, which were extracted with methanol/ultra-pure water/ formic acid after hydrolysis. Dichloromethane extracts were separated and the organic phase evaporated and reconstituted in ultra-pure water prior to quantification. Methanol/water/formic acid extracts were separated and the aqueous phased filtered prior to quantification. Residues of PHQ are quantified using *m/z* 185 to 108 as primary ions and m/z 185 to 157 as confirmatory ions (*m/z* 185 to 108 for oil).

Food of animal origin

No methods for risk assessment of OPP in animal products have been submitted under this data point. Please refer to the methods for enforcement.

Soil

No methods for risk assessment of OPP in soil have been submitted under this data point. Please refer to the methods for enforcement.

Water

For quantification of OPP in water, the following methods were developed and validated:

- HPLC-UV – Water samples were directly injected into the HPLC system. OPP was quantified at 210.4 nm or 200 nm.
- GC-MS – Water samples were filtered, adjusted to pH2 then cleaned up by solid phase extraction. The solid phase was extracted with methanol, evaporated to dryness, re-dissolved in hexane, then derivatised using diazomethane followed by acetylation of phenolic hydroxyl groups by acetohydride and triethylamine. Phosphate buffer is added, and then the mixture is partitioned with methyl-tert-butyl ether. The organic phase is dried with sodium sulphate and spiked with internal standard prior to analysis by GC-MS. The quantifier ion for OPP was 170 *m/z.*

There are no relevant metabolites for water.

Air

For quantification of OPP in air, the following method was developed and validated:

- GC-FID – Silica gel tubes used to collect air samples were extracted by agitation for 1 hour with acetonitrile. Extracts were analysed by GC with flame ionisation detection.

2.5.2 Methods for post control and monitoring purposes

Plants and plant products

For quantification of OPP in plants and plant products, the following methods were developed and validated:

- HPLC-MS/MS – Extraction of plant samples is described below:
    - Plant samples were treated with 4N HCl for acidic hydrolysis. The hydrolysed samples were treated with acidified acetonitrile, followed by extraction with magnesium sulphate, sodium chloride and citrate salts. The organic phase was cleaned up using solid phase extraction (with PSA and $MgSO_4$) then diluted with acidified acetonitrile and water prior to analysis by HPLC-MS/MS. For crop matrices with a low content of ascorbic acid, more ascorbic acid and EDTA are added for stabilisation of PHQ against oxidation during the hydrolysis step.

The quantifier ion for OPP was 169 to 115 *m/z*, the qualifier ion was 169 to 141 *m/z*.

The LOQ for OPP in citrus is 0.01 mg/kg. The LOQ for OPP in pear, oilseed rape and wheat grain is also 0.01 mg/kg.

For quantification of metabolite PHQ in plants and plant products, the same method as described above for the active substance OPP was developed and validated.

The quantifier ion for PHQ was 185 to 184 *m/z*, the qualifier ion was 185 to 108 *m/z*.

The LOQ for PHQ in citrus is 0.01 mg/kg.

Food of animal origin (foodstuff)

For quantification of OPP in food of animal origin, the following methods were developed and validated:

- HPLC-MS/MS – Extraction of different animal samples is described below:
    - Whole milk, eggs, meat, liver and fat samples were extracted with acidified acetonitrile, followed by extraction with magnesium sulphate, sodium chloride and citrate salts. The organic phase was cleaned up using solid phase extraction (after freezing for fat samples only), then diluted with acidified acetonitrile and water prior to analysis by HPLC-MS/MS.
    - Blood samples were diluted with acidified acetonitrile and water (after homogenisation for blood only) prior to analysis by HPLC-MS/MS.

The quantifier ion for OPP was 169 to 115 *m/z*, the qualifier ion was 169 to 141 *m/z*.

The LOQ for OPP in whole milk, eggs, meat, liver and fat is 0.01 mg/kg.
The LOQ of OPP in blood is 0.05 mg/L.

The residue definition includes certain metabolites.

Body fluids and tissues (toxicology)

For quantification of OPP in body fluids (human urine, bovine blood) and animal tissues (meat/muscle, liver, fat) the following methods were developed and validated:

- HPLC-MS/MS – Extraction of different samples is described below:
    - Whole meat, liver and fat samples were extracted with acidified acetonitrile, followed by extraction with magnesium sulphate, sodium chloride and citrate salts. The organic phase was cleaned up using solid phase extraction (after freezing for fat samples only), then diluted with acidified acetonitrile and water prior to analysis by HPLC-MS/MS.
    - Blood and urine samples were diluted with acidified acetonitrile and water (after homogenisation for blood only) prior to analysis by HPLC-MS/MS.

The quantifier ion for OPP was 169 to 115 *m/z*, the qualifier ion was 169 to 141 *m/z*.

The LOQ for OPP in meat, liver and fat is 0.01 mg/kg.
The LOQ of OPP in blood and urine samples is 0.05 mg/L.

The residue definition for body fluids and tissues for monitoring includes certain metabolites which will need to be further considered in the development of the method:

[…]Considering the available information, residues in body fluids and tissues could be defined as the active substance (OPP and SOPP) and its sulphate and glucuronide conjugates (major phase II metabolites), identified in urine samples of rats, collected 24 h after exposure to OPP and SOPP.[…]

Soil

For quantification of OPP in soil, the following method was developed and validated:

- HPLC-MS/MS – Soil samples were extracted with acidified acetonitrile and water in a microwave extractor for 3 minutes at 250W, then centrifuged prior to analysis by HPLC-MS/MS. The quantifier ion for OPP was 169 to 115 m/z.

The LOQ for OPP in soil is 0.005 mg/kg.

No metabolites are included in the residue definition for monitoring in soil.

Water

For quantification of OPP in water, the following method was developed and validated:

- HPLC-MS/MS – Water samples are diluted with acidified acetonitrile prior to analysis by HPLC-MS/MS. The quantifier ion for OPP was 168.9 to 115 *m/z*.

The LOQ for OPP in water is 0.1 µg/ml.

No metabolites are included in the residue definition for monitoring in water.

Air

For quantification of OPP in air, the following method was developed and validated:

- GC/MS – Tenax tubes are extracted with ethanol then analysed by GC-MS using single ion monitoring (*m/z* 115, 141, 169 and 170).

The LOQ for OPP in air is 0.35 µg/m^3.

2.6 EFFECTS ON HUMAN AND ANIMAL HEALTH

2.6.1 Summary of absorption, distribution, metabolism and excretion in mammals *[equivalent to section 9 of the CLH report template]*

Table 17: Summary table of toxicokinetic studies

Method	Results / Remarks	Reference
Excretion, distribution and metabolic fate Comparable to OECD TG 417 Rat, Fischer 344, ♂ Single oral dose of 160 mg/kg ^{14}C-OPP (5 rats) Single oral dose of 250 mg/kg ^{14}C-SOPP (4 rats) GLP: No **Supporting information**	Absorption & excretion: Relatively rapid and almost complete based on urinary and faeces excretion. 83.3% (OPP) and 85.1 % (SOPP) eliminated in urine after 24 h post-dosing. 98.2 % of OPP and 93.1 % of SOPP were recovered in urine and faeces after 7 days post-dosing. Tissue distribution: No significant retention in any organ or tissue and tissue tested after 7 days. Metabolic profile: Conjugates of OPP and PHQ with small amounts of free OPP and PHQ. Minor metabolite identified as PBQ. No remarkable difference in metabolic profile of OPP and SOPP.	Sato, M. *et al* (1988) (CA) B.6.1.1-01
Excretion, distribution and metabolic fate Comparable to OECD TG 417 Goat, Nubian, ♀ Repeat dose (5 consecutive days): 0, 13.7 mg/day or 53.3 mg/day ^{14}C-OPP (1 animal/dose) GLP: Yes **Supporting information**	Absorption & excretion: Relatively rapid and almost complete based on urinary and faeces excretion. Dose 13.7 mg/day: 94.3 % radioactive dose recovered. Dose 53.3 mg/day: 91.7 % radioactive dose recovered. Tissue distribution: No significant retention in any organ and tissue tested was apparent after 5 days Only 0.09-01 % of radioactive dose in milk. Metabolite ID: No metabolites were identified due to the low concentration of radioactive residues in the tissues.	Thalacker, F.W. (1997) (CA) B.6.1.1-02
Excretion and metabolism *in vivo* Comparable to OECD TG 417 Rat, Fischer 344, ♂ Single oral dose of 5, 50, 500 mg/kg ^{14}C-OPP (4 rats) Single oral dose of 5, 50, 500 mg/kg ^{14}C-SOPP (4 rats) Preconditioned animals: unlabelled OPP (1.3 % by weight) or SOPP (2.0 % by weight) for 2 weeks followed by single oral dose of 500 mg/kg of OPP or SOPP. *In vitro* metabolism Dose: 11 µM or 110 µM [^{14}C]-OPP System: Purified rat liver microsomes with NADPH-regenerating system GLP: No **Supporting information**	Absorption & excretion: Relatively rapid and almost complete based on urinary and faeces excretion. **500 mg/kg OPP**: 96 % excreted in urine, 6.0 % excreted in faeces. Pre-treatment experiment OPP: 88 % excreted in urine, 3.3 % in faeces. **500 mg/kg SOPP**: 91 % excreted in urine, 5.3 % in faeces. Pre-treatment experiment SOPP: 94 % excreted in urine and 5.3 % in faeces. Metabolite ID: Sulphate and glucuronide conjugates of OPP at both 5 and 50 mg/kg doses of [^{14}C]-OPP or [^{14}C]-SOPP. Sulphate and glucuronide conjugates of OPP plus conjugated PHQ at 500 mg/kg of [^{14}C]-OPP or [^{14}C]-SOPP. *In vitro* metabolism: Large amounts of material co-chomatrographed with 2,5-dihydroxybiphenyl. 33.8 % and 55.8 % of 110 µM [^{14}C]-OPP and 11 µM [^{14}C]-OPP, respectively, were converted to dihydroxybiphenyl compounds.	Reitz, R. *et al* (1983) (CA) B.6.1.1-03
Absorption, distribution, metabolism and excretion	Absorption & excretion: Relatively rapid and almost complete based on urinary	McNett, D.A. *et al*

Method	Results / Remarks	Reference
Comparable to OECD TG 417 Mice, $B_6C_3F_1$, ♂ Single oral dose 25 or 1000 mg/kg OPP (10 animals/dose) Repeat dose: 1000 mg/kg OPP (10 mice) Rat, Fischer 344 ♂/♀ Single oral dose 25 or 125 mg/kg OPP (2 animals/sex/dose) GLP: Yes **Acceptable**	and faeces excretion. Single oral dose in mice (48 h): 25 mg/kg dose group: 84 % in urine and 11 % in faeces 1000 mg/kg dose group: 98 % in urine and 6.3 % in faeces. Repeat dose in mice (48 h) 85 % in urine and 13 % in faeces (data normalised) Metabolite ID: **Mice**: Conjugates of OPP and PHQ. Low dose group OPP-S 56.3 % and OPP-G 29 %. High dose group OPP-S 21-27% and OPP-G 48-59 %. PHQ-G and PHQ-S (11 % and 23 %, respectively), not affected by dose. Minor metabolite: peak 2 (unidentified, 2 % at low dose). **Rats**: Similar profile with both doses: OPP-S (91%), OPP-G (7.1 %), PHQ-G (2.1 %), PHQ-S (1.7 %). Additionally, two minor metabolites: peak 1 (unidentified 2 %) and peak 5 (tentatively DHB-S, 2.6 %), and free OPP (0.4 %)	(1997) (CA) B.6.1.1-04
Absorption, excretion and metabolism in rat, mice, human Comparable to OECD TG 417 Mice, $B_6C_3F_1$, ♂ Single oral dose 15 or 800 mg/kg OPP (10 animals/dose) Rat, Fischer 344 ♂/♀ Single oral dose 28 mg/kg (♂) and 27 mg/kg (♀) of OPP (2 animals/sex) Humans, ♂ Dermal dose 0.006 mg/kg OPP for 8 h GLP: No **Supporting information**	Absorption and excretion: Relatively rapid and almost complete based on urinary and faeces excretion. At 48 h for mice: 84 %/98 % (low/high dose) in urine and 11 % and 6.3 % in faeces (low/high dose) At 48 h for rats: 86-89 % in urine, faeces not collected At 24 h for humans: 39 % of the applied dose or 90 % of absorbed dose Metabolite ID: **Mice**: OPP-S (57 % low dose / 21 % high dose), OPP-G (29 % low dose / 61 % high dose), PHQ-S (7.5 % low dose / 9.9 % high dose), PHQ-G (4.0 % low dose/ 8.6 % high dose). **Rats**: OPP-S (82% ♂, 86 % ♀), OPP-G (6.9 % ♂, 7.7 % ♀), PHQ-S (1.8 % ♂, 2.3 % ♀), PHQ-G (3.1 % ♂, 1.5 % ♀), DHB-S (3.0 % ♂, 1.4 % ♀), peak 1 (unknown, 3 % ♂, 1.1 % ♀). **Humans**: OPP-S (69.0 %), OPP-G (3.5 %), PHQ-G (14.5 %), DHB-S (12.5 %).	Bartels, M.J. *et al* (1998) (CA) B.6.1.1-05
Metabolite ID Rat, Fischer 344 ♂ Repeat oral doses of 0, 800, 4000, 8000 and 12500 ppm equivalent to 0, 57, 285, 568 and 937 mg/kg OPP Overnight urinary samples from weeks 12-13 GLP: Yes **Supporting information**	Metabolite ID: Metabolites: conjugates of OPP and PHQ and free OPP and PHQ. Dose-dependent OPP-S/OPP-G ratio. At lower doses OPP-S is major metabolite (OPP-S/OPP-G ratio was 67.07/12.78 at 8000ppm). Increase in OPP-G at highest dose (OPP-S/OPP-G ratio was 57.24/53.61) Levels of PHQ-S and PHQ-G increased with doses. Minor metabolites: free OPP and PHQ (levels increase with dose, 0.6-1.5 %)	Bartels, M.J. and McNett, D.A. (1996) (CA) B.6.1.1-06
Metabolite ID Dogs, Beagle (mature and inmature) 3 animals/sex/group 3.7 mg pure OPP and trace ^{14}C-OPP Cats, domestic, short-haired (mature and inmature) 3 animals/sex/group Repeat oral dose (alternate days for	Excretion: 45 % and 54 % of the administered dose was excreted in urine in puppies and adult dogs, respectively. 31 % and 42 % of the administered dose was excreted in kittens and adult cats, respectively. Metabolite ID: Puppies: OPP-G (21 %), OPP-S (8.3 %), OPP (73 %) Dogs: OPP-G (5.2 %), OPP-S (6.1 %), OPP (88.4 %) Kittens: OPP-G (0.96 %), OPP-S (3.3 %), OPP (96 %)	Savides, M.C. and Oehme, F.W. (1980) (CA) B.6.1.1-07

Method	Results / Remarks	Reference
25 days) of 3.7 mg pure OPP and trace ^{14}C-OPP, representing 2.03, 0.27, 2.04 and 1.16 mg/kg bw in puppies, dogs, kittens and cats, respectively GLP: No **Supporting information**	Cats: OPP-G (0.76 %), OPP-S (2.4 %), OPP (97 %)	
Dermal absorption Human ♂ 6 volunteers 100 µL of ^{13}C/^{14}C-OPP solution in isopropanol (0.4 % w/v) dermal application for 8 h. GLP: Yes **Acceptable**	<u>Absorption</u>: High concentrations of radioactivity in the 2 and 4 hour plasma samples indicate a rapid absorption. Mean recovery in swabs, skin rinsate, gauze and protective enclosure was 58.66 ± 11.38, indicating an absorption value of 43.15 % of applied dose. No evidence of accumulation of radioactive dose in the skin. <u>Excretion</u>: The main route of excretion is *via* urine. A mean of 42.71 ± 9.82 % of the administered radioactivity was excreted in the urine. Most of the radioactivity was excreted between 0-24 h after dosing. Minor radioactivity excreted in the faeces at a mean value of 0.45 ± 0.2 %.	Selim, S. (1996) (CA) B.6.1.2-01
Metabolite ID Human ♂ 100 µL of ^{13}C/^{14}C-OPP solution in isopropanol (0.4 % w/v) dermal application for 8 h. Urinary samples collected from the study described in B.6.1.2-01 **Acceptable**	<u>Metabolite ID</u>: The major urinary metabolite was found to the sulphate conjugate of the parent compound: OPP-S (69.0 %). The glucuronide conjugate was also identified but in minor quantities: OPP-G (3.5 %). Hydroxylated metabolites of OPP were also identified, being the glucuronide conjugate of PHQ-G (14.5 %) and the sulphate conjugate of DHB-S (12.5 %). Free OPP was only detected in urine collected at early hours post-dosing (0-4 h) and accounted for 0.5 %.	Bartels, M.J. *et al* (1997) (CA) B.6.1.2-02 (AS)
Pharmacokinetic modelling Human ♂ 100 µL of ^{13}C/^{14}C-OPP solution in isopropanol (0.4 % w/v) dermal application for 8 h. Urinary samples collected from the study described in B.6.1.2-01 No guideline **Supporting information**	One compartment model. Absorption of 43 % of applied dose. Absorption half-life of 10 ± 2 h. Rapid clearance, primarily *via* urine, elimination half-life of 0.8 ± 0.1 h. Volume of distribution (V_d) was 15 ± 3.0 mL/ Model parameters in agreement with experimental data.	Timchalk, C. (1996) (CA) B.6.1.2-03
In vitro and *in vivo* percutaneous absorption OECD TG 427, OECD TG 428 Vehicle: 60 % aqueous ethanol Skin samples (*in vitro* studies): Human ♀ Rat Wistar and Sprague-Dawley ♂ Dose: 120 µg ^{14}C-OPP /cm^2 (= 2.63 µCi/cm^2) *In vivo* studies : Rat Wistar albino ♂ (4 animals) Dermal dose : 100 µL/250 g bw (250	**Human volunteers**: Percutaneous absorbed dose: 105 ± 9 µg Maximal flux 11.0 ± 4.11 µg/cm^2/h Kp value 15.8 ± 5.9x10^{-3} cm/h Urinary excretion of OPP (parent+metabolites) was 14.9 ± 2.5 % of applied dose (dermal) Urinary excretion of OPP (parent + metabolites) was 60.5 ± 8.8 % after iv dose **Human *in vitro*:** Absorption: 32.9 ± 4.9 % Maximal flux 1.11 ± 0.39 µg/cm^2/h Kp value 1.59 ± 0.56 x10^{-3} cm/h **Rat *in vivo***:	Cnubben *et al* (2002) (CA) B.6.1.2-04

Method	Results / Remarks	Reference
µCi/mL) Iv dose : 25.2 µg ^{14}C-OPP/ mL dosed at 2 mL/kg bw. Human ♂ (caucasian) Dermal dose : 0.3 mL of OPP (40 mg/mL) for 4 h. iv dose : 2.5 mg/250 mL ethanol/saline **Supporting information**	Maximal flux 27.5 ± 10.3 µg/cm^2/h Kp value 39 ± 15 x10^{-3} cm/h Urinary excretion of OPP (parent+metabolites) was 37.8 ± 2.7 % of applied dose (dermal) Urinary excretion of OPP (parent + metabolites) was 88.6 ± 8.5 % after iv dose Excretion in faeces was 2.2 % (iv) and less than 1 % (dermal) **Rat *in vitro***: Absorption: 23.6 ± 2.3 % Maximal flux 0.68 ± 0.08 µg/cm^2/h Kp value 0.97 ± 0.11 cm/h Overall the *in vivo* absorption characteristics of OPP in rats slightly overpredicted the human situation with a factor of 1.5 to 2.5 based on Kp values and systemically available.	

2.6.1.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The applicant has submitted a total of eleven studies for the renewal of approval of the substance *ortho*-phenylphenol, all of which were evaluated in the original DAR (2008). Only two studies out of the eleven also included ADME studies for sodium *ortho*-phenylphenol (mainly absorption, excretion and metabolite ID). No comparative *in vitro* metabolism study in various species has been provided although the metabolism data from human studies are considered sufficient to establish the comparison and equivalence.

Ortho-Phenylphenol (OPP) and the sodium salt (SOPP) are rapidly and almost totally absorbed based on urinary and faeces excretion following oral administration in rats and mice.

In rats, single oral doses of 160 mg/kg ^{14}C-OPP or 250 mg/kg ^{14}C-SOPP (B.6.1.1-01) resulted in the elimination of 83.3 % and 85.1 %, respectively, of the applied radioactive dose in urine 24 h post-dose. Recovery in urine and faeces after 7 days accounted for 98.2 % of ^{14}C-OPP and 93.1 % ^{14}C-SOPP (B.6.1.1-01).

In mice, single oral doses of 25 or 1000 mg/kg bw ^{14}C-OPP resulted in a total recovery of applied radioactive dose after 48 h of 84 % and 98 % in urine, respectively, and 11.2 % and 6.3 % in faeces, respectively (B.6.4.1.1-04). Similar results were obtained in mice orally dosed 15 or 800 mg/kg bw ^{14}C-OPP (B. 6.4.1.1-05).

No significant retention in any organ and tissue tested was apparent in rats (B.6.1.1-01) and mice.

The majority of OPP and SOPP administered to rats and mice undergo immediate phase-II metabolism, and are excreted as sulphate or glucuronide conjugates. Minute amounts of unconjugated parent compound were recovered from urine. None of the studies submitted had identified metabolites in faeces.

A total of 8-radiolabelled metabolites were detected and identified in rats and mice urine following oral exposure to OPP (B.6.1.1-05). The profile for metabolites present in the urine of male and female rat administered a low dose of OPP was comparable (B.6.1.1-05). The sulphate conjugate of OPP (OPP-S) was the major radiolabelled compound found in urine followed by the glucuronide conjugate of OPP (OPP-G). Lesser amounts of glucuronide (PHQ-G) and sulphate (PHQ-S) conjugates of phenylhydroquinone (PHQ) were present. Two minor metabolites of OPP were also observed, one of them was not identified and the other was found to be the sulphate conjugate of 2,4'-dihydroxybiphenyl (2,4'-DHB-S).

In male rats, repeat oral doses of OPP (0, 57, 285, 568 and 937 mg/kg bw) showed a shift in the ratio of sulphate vs glucuronide conjugates of OPP; the sulphation pathway for OPP appears to saturate at high subchronic dietary doses and glucuronidation and PHQ formation (excreted as sulphate and glucuronide conjugates) becomes more significant (B.6.1.1-06).

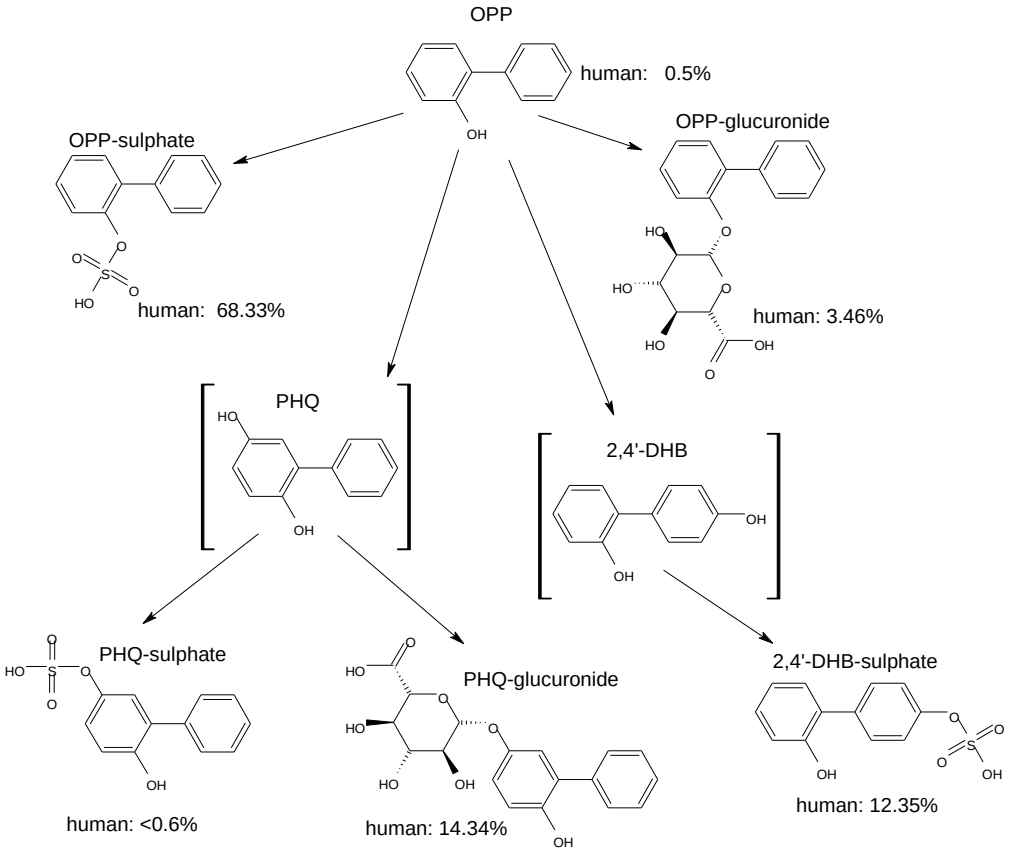
In male mice at low dose, the majority of oral dose was found as the sulphate and glucuronide conjugates of the

OPP in urine (B.6.1.1-05). PHQ-S and PHQ-G were also found as minor metabolites in the mouse. An additional polar metabolite was observed but no characterised. No free OPP, PHQ or DHB was found in the urine of mice. The metabolic fate of OPP in the mouse was found to change with increasing dose. At high doses, the conjugates of parent OPP still accounted for the majority of the administered dose, however sulphation of OPP was apparently saturated, and a corresponding increase in OPP-G was observed. The amount of test material metabolised *via* hydroxylation to PHQ also increased with dose in the mouse.

Cats and dogs appear to excrete the majority of orally administered OPP (7 weeks) as the un-metabolised parent compound (B.6.1.1-07).

$^{13}C/^{14}C$-OPP solution in isopropanol was dermally applied to human volunteers (B.6.1.2-01, B.6.1.2-02). The sulphate conjugate of OPP was the major metabolite in the urine (69 %) with low level of OPP-G (3.5 %) (Figure 2.6.1.1/1). Hydroxylated metabolites such as PHQ-G (14.5 %) and 2,4'-DHB-S (12.5 %) were also identified urinary metabolites. Unlike in rat and mouse, no PHQ-Sul was found as a human metabolite of OPP.

Figure 2.6.1.1/1: Structures and abundance of urinary metabolites of OPP found in human following dermal exposure to *ortho*-phenylphenol for 4 h (data from study B.6.1.2-02).



Conclusion:

Data available for OPP and SOPP suggests both substances may have similar absorption, distribution and excretion behaviours. The metabolic profile of both compounds is reported to have no remarkable differences (B.6.1. 1-01 and B.6.1.1-03) and therefore, the metabolism is deemed equivalent. Similarly, data available on metabolism profile of OPP and SOPP across species indicate the major metabolites identified are Phase II conjugates (glucuronide and sulphate) of the parent compound and to a lesser extent, conjugates (glucuronide and sulphate) of phenylhydroquinone (PHQ), all of which were detected in humans, rats and mice.

Residue definition for body fluids and tissues:

Considering the available information, residues in body fluids and tissues could be defined as the active substance (OPP and SOPP) and its sulphate and glucuronide conjugates (major phase II metabolites), identified in urine samples of rats, collected 24 h after exposure to OPP and SOPP.

2.6.2 Summary of acute toxicity

2.6.2.1 Acute toxicity - oral route [equivalent to section 10.1 of the CLH report template]

Table 18: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations[1] if any	**Species, strain, sex, no/group**	**Test substance, dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
Acute oral toxicity study in rats Prior to OECD TG 401 GLP: No (prior to GLP enforcement) Deviations: Test material no characterised. Animals were not fasted; Dosing into duodenum; Necropsy: by random sample; Individual body weights not reported. **Supportive only**	Species: Rat Strain: Wistar 10 male rats/group	*ortho*-phenylphenol (**OPP**) Purity: not indicated Vehicle: Lutrol (polyethylene glycol) Oral (dosing into duodenum) Single dose Doses: 1500, 2000, 3100, 4000, 4500 and 5000 mg/kg bw. 14-day observation period	Mortality: Dose mg/kg bw / Males Mortality 1500: 0/10 2000: 4/10 3100: 4/10 4000: 6/10 4500: 8/10 5000: 10/10 Clinical signs: anaesthesia, impaired general condition, abdominal recumbency, lateral recumbency Necropsy (random): No macroscopic findings **OPP LD_{50} = 2980** mg/kg bw (male rats)	Löser, E. (1981) (CA) B.6.2.1-01
Acute oral toxicity study in rats Prior to OECD TG 401 GLP: No (prior to GLP enforcement) Deviations: Only brief summary written in German. Test substances not characterised; strain, sex and weight of test animals not reported; animals were not fasted; 7 days observation period; necropsy not performed. **Supportive only**	Species: Rat Strain: not indicated 15 male rats/group	o-oxydiphenyl (**OPP**) and m-oxydiphenyl Purity: not indicated Vehicle: Lutrol (polyethylene glycol) Oral gavage Single dose Doses: 500, 1000 and 2500 mg/kg bw. 7-day observation period	Mortality: Not occurred. Clinical signs: not observed. Necropsy: not performed. **OPP LD_{50}: > 2500** mg/kg bw (male rats)	Kimmerle, G. and Lorke, D. (1969) (CA) B.6.2.1-02
Acute oral toxicity study in rats Prior to OECD TG 401 (1987) GLP: Not applicable. Published study Deficiences: only a brief summary. Batch of the test substance not reported; strain of	Species: Rat Strain: not indicated 10-20 male rats/group	*ortho*-phenylphenol (**OPP**) Purity: >98% Vehicle: olive oil/gum acacia Oral gavage Single dose Doses: 1600, 2000, 2400, 2800, 3000, 3200 and 4000 mg/kg bw.	Mortality: Dose mg/kg bw / Males Mortality 1600: 0/10 2000: 4/20 2400: 5/20 2800: 8/10 3000: 6/10 3200: 6/10 4000: 16/19 Clinical signs: not observed. Necropsy: not performed.	Hodge, H.C. *et al.* (1952) (CA) B.6.2.1-03

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Value LD_{50}	Reference
animals not specified; incomplete test method description; individual body weights only recorded at the beginning of the study; necropsy not performed. **Supportive only**		14-day observation period	**OPP LD_{50} = 2700** mg/kg bw (male rats)	
Acute oral toxicity study in mice Not possible to check test method. GLP: Not applicable. Published study Deviations: publication written in Japanese. Only abstract and results table/graphs are written in English. It is not possible to check the method. Purity of test substance not reported. **Supportive only**	Species: Mouse Strain: ddY 10 mice/sex/group	*ortho*-phenylphenol (**OPP**) Purity: not indicated Vehicle: propylene glycol Oral gavage Single dose Doses: 0, 414, 538, 700, 910, 1183, 1538 and 2000 mg/kg bw. 14-day observation period	Mortality: Dose mg/kg bw / Mortality Males / Mortality Females 0 / 0/10 / 0/10 414 / 0/10 / 0/10 538 / 0/10 / 0/10 700 / 1/10 / 0/10 810 / 0/10 / 0/10 1183 / 5/10 / 6/10 1538 / 7/10 / 10/10 2000 / 10/10 / 10/10 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. **OPP LD_{50} = 1200** mg/kg bw (male mice) **OPP LD_{50} = 1050** mg/kg bw (female mice)	Taniguchi, Y. *et al.* (1981) (CA) B.6.2.1-04
Acute oral toxicity study in rats OECD TG 401 (1987) GLP: Yes **Study acceptable**	Species: Rat Strain: Fischer 344 5 animals/sex/dose group	*ortho*-phenylphenol (**OPP**) Purity: 99.9% Vehicle: corn oil Oral gavage Single dose Doses: 500, 2500 and 5000 mg/kg bw 14-day observation period	Mortality: Dose mg/kg bw / Mortality Males / Mortality Females 500 / 0/5 / 0/5 2500 / 2/5 / 2/5 5000 / 5/5 / 5/5 Clinical signs: observed at 2500 and 5000 mg/kg bw: lacrimation, salivation, chromorhinorrhea, laboured respiration, decreased activity, lateral recumbency and urine and faecal soiling in the perineal area. Body weight: All surviving animals gained weight during the observation period. Necropsy: 500 mg/kg bw: no findings; 2500 mg/kg bw: hemolysed blood in the digestive tract (dead on day 2) and perineal soiling (dead on day 3); Surviving males at 2500 mg/kg bw: fibrous adhesions between the serosa of the non-glandular portion of the stomach and liver; surviving females at	Gilbert, K.S. and Crissman, J.W. (1994) (CA) B.6.2.1-05

<table>
<tr><th>Method, guideline, deviations[1] if any</th><th>Species, strain, sex, no/group</th><th>Test substance, dose levels, duration of exposure</th><th>Value
LD_{50}</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>2500 mg/kg bw: no gross lesions.
5000 mg/kg bw: all animals dead on day 1 (5 females, 2 males) had no gross lesions; Animals dead on day 2: hemolysed blood in the digestive tract; Animals dead on day 3: perineal soiling and lung congestion lesions

OPP LD_{50} = 2733 mg/kg bw (both sexes)</td><td></td></tr>
<tr><td>Acute oral toxicity study in mice
Not possible to check test method.
GLP: Not applicable.
Published study
Deficiencies: publication written in Japanese. Only brief abstract and results table/graphs are written in English. It is not possible to check the method.
Supportive only</td><td>Species: Mouse
Strain: IRC
10 mice/sex/group</td><td>ortho-phenylphenol (OPP)
Purity: 98%
Vehicle: olive oil
Oral
Single dose
Doses: 1000, 1500, 2250, 3375, 5063 and 7594 mg/kg bw
14-day observation period</td><td>Mortality:<table><tr><th rowspan="2">Dose mg/kg bw</th><th colspan="2">Mortality</th></tr><tr><th>Males</th><th>Females</th></tr><tr><td>0</td><td>0/10</td><td>0/10</td></tr><tr><td>1000</td><td>0/10</td><td>0/10</td></tr><tr><td>1500</td><td>0/10</td><td>0/10</td></tr><tr><td>2250</td><td>2/10</td><td>3/10</td></tr><tr><td>3375</td><td>4/10</td><td>7/10</td></tr><tr><td>5063</td><td>8/10</td><td>8/10</td></tr><tr><td>7594</td><td>10/10</td><td>9/10</td></tr></table>Clinical signs: Decrease of motor activity, sedation and lacrimation were the main clinical symptoms.

OPP LD_{50} = 3499 mg/kg bw (male mice)
OPP LD_{50} = 3152 mg/kg bw (female mice)</td><td>Tayama, K. et al. (1983) (CA) B.6.2.1-06</td></tr>
<tr><td>Acute oral toxicity study in rats
OECD TG 401 (1987)
GLP: Yes
Study acceptable</td><td>Species: Rat
Strain: Fischer 344
5 animals/sex/dose group</td><td>Sodium ortho-phenylphenate (SOPP)
Purity: 99.1%
Vehicle: 0.5% methocel
Oral gavage
Single dose
Doses: 100, 500, 1000 and 5000 mg/kg bw
14-day observation period</td><td>Mortality:<table><tr><th rowspan="2">Dose mg/kg bw</th><th colspan="2">Mortality</th></tr><tr><th>Males</th><th>Females</th></tr><tr><td>100</td><td>0/5</td><td>0/5</td></tr><tr><td>500</td><td>1/5</td><td>2/5</td></tr><tr><td>1000</td><td>3/</td><td>4/5</td></tr><tr><td>5000</td><td>5/5</td><td>5/5</td></tr></table>Clinical signs: lacrimation, salivation, chromorhinorrhea, laboured respiration, decreased activity, lateral recumbency, incoordination, decreased muscle tone, mouth breathing and urine and fecal soiling in the perineal area.
Body weight: Most surviving rats gained weight during the observation period, with the exception of one female from the 500 mg/kg bw dose group that lost weight, and had a persistence of numerous clinical signs throughout the observation period.
Necropsy: animals died during the study showed treatment-related gross pathologic observations consisting of one or more of the following findings: hemolyzed blood in the digestive tract, perineal soiling, general visceral congestion, decreased amount of fat, pale liver, congested lungs, bloody urine and congestion, erosions and/ or ulcers, hemorrhage, or hyperemia of the stomach. The gross observations of</td><td>Gilbert, K.S. and Stebbins, K.E. (1994) (CA) B.6.2.1-07</td></tr>
</table>

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Value LD_{50}	Reference
			the stomach and digestive tract were consistent with stress-induced alterations. **SOPP LD_{50} = 591** mg/kg bw (males) **SOPP LD_{50} = 846** mg/kg bw (females)	
Acute oral toxicity study in rats Prior to OECD TG 401 GLP: No (prior to GLP enforcement) Deviations: Animals were not fasted, test material not characterised, necropsy was not performed. Individual body weights were not reported. **Supportive only**	Species: Rat Strain: Wistar 5 rats/sex/group	Sodium *ortho*-phenylphenate (**SOPP**) Purity: not indicated Vehicle: Water Oral gavage Single dose Doses: 1000, 1300, 1500, 2000, 2200 and 2500 mg/kg bw. 14-day observation period	Mortality: Dose mg/kg bw / Mortality: Males / Females 1000: 0/5 / 0/5 1300: 1/5 / 1/5 1500: 1/5 / 3/5 2000: 2/5 / 2/5 2200: 4/5 / 5/5 2500: 5/5 / 5/5 Clinical signs: narcosis and a decline in general conditions Necropsy: not performed. **SOPP LD_{50} = 1720** mg/kg bw (combined)	Löser, E. (1980) (CA) B.6.2.1-08

Table 19: Summary table of human data on acute oral toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 20: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

2.6.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

All the available acute oral toxicity studies performed with ***ortho*-phenylphenol (OPP)** were included and assessed in the previous DAR (2008). Only one of these six studies clearly complies with the guidance test methods. The resulting LD_{50} of this acute oral toxicity study (Gilbert, K.S. and Crissman, J.W., 1994; B.6.2.1-05) is 2733 mg/kg bw for male and female rats.

The other five studies presented significant deficiencies (e.g. test substance not characterized, dosing into duodenum, observation period excessively short, or the provided reports consisted in brief summaries or publications with lack of data and/or not translated, so it was not possible to check the method) and therefore these studies are considered as supportive, but not acceptable for classification purposes.

The results of four of these supportive studies, are in line with the accepted one, with LD_{50} values greater than 2000 mg/kg bw (ranging from >2500 to 3499 mg/kg bw), in both rats and mice.

On the contrary, the results of the acute oral toxicity study in ddY mice (Taniguchi, Y. *et al.*, 1981; B.6.2.1-04), showed a LD_{50} of 1050 mg/kg bw for females and 1200 mg/kg bw for males. However, several uncertainties arise from the provided report (a Japanese publication where only the summary is written in English), like the unknown characterisation of the test substance, the conditions of the study or the lack of justification of the selection of ddY mice strain, and its implications on the assessment of the test results for classification purposes.

Due to the uncertainties aroused from this study report, and considering that the results of the other study in a different strain of mice (LD_{50} of 3152 mg/kg bw for female and 3499 mg/kg bw for male IRC mice) are congruent

with the results obtained in the other 4 studies (included the acceptable one), the overall conclusion is that *ortho*-phenylphenol shows low acute oral toxicity, with an oral LD_{50} greater than 2500 mg/kg bw.

ortho-Phenylphenol classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). No classification regarding acute oral toxicity is included.

Regarding the data available on **sodium *ortho*-phenylphenate (SOPP)**, two new studies have been included for the renewal assessment of the active substance *ortho*-phenylphenol. No studies on acute oral toxicity of SOPP were included in the previous DAR (2008).

The results obtained in both studies show evidence of acute oral toxicity of SOPP, with a LD_{50} between 500 and 2000 mg/kg bw. However, only one of these two studies is considered acceptable (Gilbert, K.S. and Stebbins, K.E., 1994; B.6.2.1-07) and, therefore, the result obtained in this study is the one considered for the assessment of the acute oral toxicity of SOPP: ATE = 591 mg/kg bw (LD_{50} = 591 mg/kg bw (males) and LD_{50} = 846 mg/kg bw (females)).

Sodium *ortho*-phenylphenate classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). Classification regarding acute oral toxicity is included as: Acute (oral) toxicity, category 4 (Acute Tox. 4*; H302).

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

The LD_{50} of ***ortho*-phenylphenol (OPP)** is 2733 mg/kg bw (according to the study B.6.2.1-05), which is above the threshold value of 2000 mg/kg bw for triggering acute oral toxicity classification.

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

Data available indicates that ***ortho*-phenylphenol (OPP)** does not require classification for acute oral toxicity, according to Regulation (EC) No. 1272/2008.

2.6.2.2 Acute toxicity - dermal route [equivalent to section 10.2 of the CLH report template]

Table 21: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations[1] if any	**Species, strain, sex, no/group**	**Test substance, dose levels, duration of exposur**	**Value LD_{50}**	**Reference**
Acute dermal toxicity study in rats OECD TG 402 (1987) GLP : Yes **Study acceptable**	Species: rat Strain: Wistar 5 rats/sex	*ortho*-phenylphenol (**OPP**) Purity: 99.89% Vehicle: Cremophor E Dermal Single dose Dose: 2000 mg/kg bw Dose: 2000 mg/kg bw 24-h exposure 14-day observation period	Mortality: not occurred Clinical signs: Slight reddening of 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 **OPP LD_{50} > 2000** mg/kg bw (both sexes)	Bomhard, E. (1991) (CA) B.6.2.2-01
Acute dermal toxicity study in rabbits OECD TG 402 (1981) GLP : No	Species: rabbit Strain: New Zealand White 2 rabbits/sex	*ortho*-phenylphenol (**OPP**) Purity: 99.73% Dermal: applied dry on the skin.	Mortality: not occurred Clinical signs: Lethargy following treatment. Topical responses included slight to moderate erythema and oedema and marked necrosis at the application site. Body weight: One female showed a decrease	Carreon, R.E. and New, M.A. (1981) (CA) B.6.2.2-02

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposur	Value LD_{50}	Reference
Deviations: Only 2 animals per sex were used. **Supportive only**		Water was added to simulate moistened skin. Single dose Dose: 5000 mg/kg bw 24-h exposure 14-day observation period	in body weight at the end of the study. Necropsy: No treatment-related effects **OPP LD_{50} > 5000** mg/kg bw (both sexes)	
Acute dermal toxicity study in rats OECD TG 402 (1987) GLP : Yes **Supplementary only**	Species: rat Strain: Wistar 5 rats/sex	Sodium *ortho*-phenylphenate (**SOPP**) Purity: not indicated Dermal Single dose Dose: 2000 mg/kg bw 24-h exposure 14-day observation period	**No dermal LD_{50} value** could be established **for SOPP,** due to its corrosive properties All animals died during the first 5 days of the study: one was found dead on day 5 and the others were sacrified for humane reasons after considering the severity of the necrosis produced by the substance. The death of the animal that died was also considered related to necrosis.	Busschers, M. (1997) (CA) B.6.2.2-03

Table 22: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 23: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

2.6.2.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

Two acute dermal toxicity studies were included for the assessment of ***ortho*-phenylphenol (OPP)**. These studies were already assessed in the previous DAR (2008). One of these two studies (Bomhard, E., 1991; B.6.2.2-01) complies with the guidance test methods, and no deviation from the guideline was observed. The resulting LD_{50} of this study is > 2000 mg/kg bw for male and female rats.

The other study (Carreon, R.E. and New, M.A., 1981; B.6.2.2-02) is considered as supportive, since only 2 animals per sex were used. The results of this study are in line with the acceptable one, since shows low acute dermal toxicity of *ortho*-phenylphenol (LD_{50} > 50000 mg/kg bw).

ortho-Phenylphenol classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). No classification regarding acute dermal toxicity is included.

Regarding the data available on **sodium *ortho*-phenylphenate (SOPP)**, a new study (Busschers, M., 1997; B.6.2.2-03) has been included for the renewal assessment of the active substance. No studies on acute dermal toxicity of SOPP were included in the previous DAR (2008).

In this study, the severe necrosis produced by SOPP derived in the death of one animal and the sacrifice for human reasons of the other 9 animals of the study. According to the test method OECD TG 402, the acute dermal toxicity test should not be carried out with corrosive substances. Therefore, no LD_{50} value could be derived in this study, nor should it be tested again.

Sodium *ortho*-phenylphenate classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). No classification regarding acute dermal toxicity is included.

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

The LD_{50} of ***ortho*-phenylphenol (OPP)** is greater than 2000 mg/kg bw (according to the study B.6.2.2-01), which is above the threshold value of 2000 mg/kg bw for triggering acute dermal toxicity classification.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

Data available indicates that ***ortho*-phenylphenol (OPP)** does not require classification for acute dermal toxicity.

2.6.2.3 Acute toxicity - inhalation route [equivalent to section 10.3 of the CLH report template]

Table 24: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value LC_{50}**	**Reference**
Acute inhalation toxicity study in rats OECD TG 403 (1981) GLP : Yes **Study acceptable**	Species: rat Strain: Fischer 344 5 rats/sex	*ortho*-Phenylphenol (**OPP**). Purity: 99.8% Test atmosphere: **Parameter** / **Value** Nominal concentration: 13.00 mg/L Mean max. attainable concentration: 0.036 mg/L Particles < 1 µm: > 50% Chamber temperature: 22.6 ± 0.5 ºC Chamber relative humidity: 45.1 ± 6.7 % Air flow rate: 30 ± 0 L/min MMAD and GSD were not calculated because the particle size distribution was not log-normal. No mortality occurred 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 (14 d). Necropsy: No abnormalities were noted.	Dose: 0.036 mg/L (Max.attainable concentration) Exposure: 4-h (nose-only)	**>0.036 mg/L/4h** No mortality	Landry, T.D., Stebbins, K.E. and Battjes, J.E. (1992) (CA) B.6.2.3-01
Acute inhalation toxicity study in rats Prior to OECD TG 403 (1981) GLP: No Deviations: Test substance and test atmosphere not characterized. Exposure time: only 1 h. Observation period only 7 d. **Supportive only**	Species: rat Strain: Wistar II 20 male rats/group	Test substances: *ortho*-Phenylphenol (**OPP**) and sodium *ortho*-phenylphenate (**SOPP**) Test atmosphere not characterized	Doses: OPP: 0.228, 0.447 and 0.949 mg/L air SOPP: 1.331 mg/L air Exposure: 1-h (via inhaled air)	**OPP: >0.949 mg/L/1h** **SOPP: >1.331 mg/L/1h**	Mihail, F. and Kimmerle, G. (1977) (CA) B.6.2.3-02

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation toxicity study in rats Time-saturation test No guideline (similar to OECD TG 403, 1981)-Annex: Inhalation hazard test GLP : No Deviations: 7 h exposure. Batch and test article preparation not reported. The concentration of the test substance was not measured. Information of exposure parameters and individual body weights were not reported. **Supportive only**	Species: rat Strain: Wistar 5 rats/sex	*Ortho*-Phenylphenol (**OPP**). Purity: >99.5% Test atmosphere not characterized.	Doses: not determined (air enriched with vapour of OPP) Exposure: 7-h (whole body)	**Not determined**	Thyssen, J., (1982) (CA) B.6.2.3-03

Table 25: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 26: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

2.6.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Three acute inhalation toxicity studies were included for the assessment of ***ortho*-phenylphenol (OPP)**. These studies were already assessed in the previous DAR (2008). One of these three studies (Landry, T.D., Stebbins, K.E. and Battjes, J.E., 1992; B.6.2.3-01) complies with the guidance test methods, and no deviation from the guideline was observed. The resulting LC_{50} of this study is > 0.036 mg/L (maximum attainable concentration) for male and female rats.

The other two studies are considered as 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 (Mihail, F. and Kimmerle, G., 1977; B.6.2.3-02) or 7 hours (Thyssen, J., 1982; B.6.2.3-02) instead of 4 hours.

ortho-Phenylphenol classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). No classification regarding

acute inhalation toxicity is included.

Regarding the **sodium *ortho*-phenylphenate (SOPP)**, only the study of Mihail, F. and Kimmerle, G., 1977 (B.6.2.3-02) is available. This study was already assessed in the previous DAR (2008), since both substances (OPP and SOPP) were included in the study.

As previously commented for OPP, this study is considered as supportive only, due to deviations from the method, where atmosphere was not characterized and the exposure time was of 1 hour. Therefore, the LC_{50} value of >1.331 mg/L/1h, is not considered suitable for classification.

No other information is available in the dossier to assess the classification of SOPP regarding acute inhalation toxicity.

Moreover, sodium *ortho*-phenylphenate classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). No classification regarding acute inhalation toxicity is included.

2.6.2.3.2 Comparison with the CLP criteria regarding acute inhalation toxicity

ortho-Phenylphenol (OPP): the four-hour inhalation study in rats reported an $LC_{50} \geq 0.036$ mg/L (maximum attainable concentration).

According to the classification criteria under Regulation (EC) No. 1272/2008 the threshold for no classification for acute inhalation toxicity is an $LC_{50} > 5$ mg/L for dusts or mists. However, considering that the maximum attainable concentration did not produce any mortality, no classification for acute inhalation toxicity is therefore proposed.

2.6.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Data available indicates that ***ortho*-phenylphenol (OPP)** does not require classification for acute inhalation toxicity.

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

		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	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.	Hodge *et al.* 1952 B.6.2.1-03 Published study

Supportive study	14-day observation period	**LD_{50} = 2700 mg/kg bw**	
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 **Key study**	Purity: 99.8% Nominal concentration: 13.00 mg/L Mean max. attainable Concentration: 0.036 mg/L 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)	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 **LC_{50} >0.036 mg/L/4 h**	B.6.2.3-01, 1992
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**.

2.6.2.4 Skin corrosion/irritation [equivalent to section 10.4 of the CLH report template]

Table 27: Summary table of animal studies on skin corrosion/irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group,</th><th>Test substance, dose levels, duration of exposure</th><th>Results
- Observations and time point of onset
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr>
<td>Skin irritation/ corrosion in rabbits
OECD TG 404 (1987)
GLP: Yes

Study acceptable</td>
<td>Species: rabbit
Strain: New Zealand White
3 rabbits/sex</td>
<td>ortho-Phenylphenol (OPP) Purity: 99.9%
Dose: 0.5 g Applied moistened with water (0.3 ml)

4 h exposure</td>
<td>Results:
<table>
<tr><th rowspan="3">Obs. time</th><th colspan="6">Male rabbit No.</th><th colspan="6">Female rabbit No.</th></tr>
<tr><th colspan="2">0</th><th colspan="2">1</th><th colspan="2">2</th><th colspan="2">3</th><th colspan="2">4</th><th colspan="2">5</th></tr>
<tr><th>E*</th><th>O*</th><th>E</th><th>O</th><th>E</th><th>O</th><th>E</th><th>O</th><th>E</th><th>O</th><th>E</th><th>O</th></tr>
<tr><td>30 min</td><td>1</td><td>0</td><td>0</td><td>0</td><td>4#</td><td>4</td><td>4#</td><td>1</td><td>4#</td><td>1</td><td>4#</td><td>2</td></tr>
<tr><td>24 h</td><td>1</td><td>0</td><td>1</td><td>0</td><td>4#</td><td>4</td><td>4#</td><td>1</td><td>4#</td><td>1</td><td>4#</td><td>2</td></tr>
<tr><td>48 h</td><td>0</td><td>0</td><td>0</td><td>0</td><td>4#</td><td>4</td><td>4#</td><td>2</td><td>4#</td><td>0</td><td>4#</td><td>2</td></tr>
<tr><td>72 h</td><td>0</td><td>0</td><td>0</td><td>0</td><td>4#</td><td>4</td><td>4#</td><td>2</td><td>4#</td><td>0</td><td>4#</td><td>2</td></tr>
<tr><td>7 d</td><td>0</td><td>0</td><td>0</td><td>0</td><td>4◊</td><td>0</td><td>4◊</td><td>0</td><td>4◊</td><td>0</td><td>4◊</td><td>0</td></tr>
<tr><td>15 d</td><td>0</td><td>0</td><td>0</td><td>0</td><td>4Δ</td><td>0</td><td>4Δ</td><td>0</td><td>4Δ</td><td>0</td><td>4Δ</td><td>0</td></tr>
<tr><td>Mean 24/48/72 h</td><td>0.3</td><td>0.0</td><td>0.3</td><td>0.0</td><td>4.0</td><td>4.0</td><td>4.0</td><td>1.7</td><td>4.0</td><td>0.3</td><td>4.0</td><td>2.0</td></tr>
<tr><td>Reversible</td><td>Y</td><td>–</td><td>Y</td><td>–</td><td>N</td><td>Y</td><td>N</td><td>Y</td><td>N</td><td>Y</td><td>N</td><td>Y</td></tr>
</table>
* E: erythema, O: oedema
# Burns observed at application site.
◊ Scabs observed at application site.
Δ Scars observed at application site.
Two males showed no oedema formation. Slight to moderate oedema was recorded at 30 min, and persisted for 24 h, in the 3 females; the application site appeared normal at 48 h for 1 female and on day 7 for the other 2. Severe oedema was seen in 1 male</td>
<td>Gilbert, K.S. (1994a) (CA) B.6.2.4-01</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group,</th><th>Test substance, dose levels, duration of exposure</th><th>Results
- Observations and time point of onset
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>at 30 min after the patch removal, and persisted for 72 h.
Oedema was resolved in all animals within 7 days.
Very slight erythema was observed at the application site of 2/6 animals 24 hours after patch removal. The other 4 animas showed burns within 30 min after the patch removal that persisted for 72 h, turned into scabs on days 7 to 10 and were recorded as scars at application site on test day 15 (end of the study).</td><td></td></tr>
<tr><td>Skin irritation/ corrosion in rabbits
OECD TG 404
GLP: No
Deviations: Exposure conditions not reported. The study finalised after 7 d (insufficient to evaluate the reversibility of the effects).
First scoring at 2 h instead of 60 min; batch and test article preparation, and individual body weights, not reported.
Supportive only</td><td>Species: rabbit
Strain: New Zealand White
6 rabbits (both sexes)</td><td>ortho-Phenylphenol (OPP)
Purity: >99.5%
Dose: not described

4h exposure.</td><td>Results:
<table><tr><th rowspan="2">Obs. time</th><th colspan="12">Rabbit No.</th></tr><tr><th colspan="2">102</th><th colspan="2">101</th><th colspan="2">97</th><th colspan="2">95</th><th colspan="2">94</th><th colspan="2">83</th></tr><tr><th>E*</th><th>O*</th><th>E</th><th>O</th><th>E</th><th>O</th><th>E</th><th>O</th><th>E</th><th>O</th><th>E</th><th>O</th></tr><tr><td>2 h</td><td>1</td><td>0</td><td>1</td><td>1</td><td>1</td><td>0</td><td>3</td><td>0</td><td>1</td><td>0</td><td>4</td><td>1</td></tr><tr><td>24 h</td><td>0</td><td>0</td><td>4</td><td>2</td><td>2</td><td>1</td><td>4</td><td>2</td><td>1</td><td>0</td><td>4</td><td>1</td></tr><tr><td>48 h</td><td>4</td><td>0</td><td>4</td><td>2</td><td>1</td><td>0</td><td>1</td><td>4</td><td>3</td><td>1</td><td>4</td><td>1</td></tr><tr><td>72 h</td><td>4</td><td>0</td><td>4</td><td>1</td><td>0</td><td>0</td><td>4</td><td>1</td><td>3</td><td>1</td><td>4</td><td>1</td></tr><tr><td>7 d</td><td>4</td><td>0</td><td>4</td><td>1</td><td>0</td><td>0</td><td>4</td><td>1</td><td>3</td><td>1</td><td>4</td><td>1</td></tr><tr><td>Mean 24/48/72h</td><td>2.7</td><td>0</td><td>4</td><td>1.7</td><td>1</td><td>0.3</td><td>3</td><td>2.3</td><td>2.3</td><td>0.7</td><td>4</td><td>1</td></tr><tr><td>Reversible</td><td>N</td><td>–</td><td>N</td><td>N</td><td>Y</td><td>Y</td><td>N</td><td>N</td><td>N</td><td>N</td><td>N</td><td>N</td></tr></table>* E: erythema, O: oedema
The skin lesions observed 72 hours after the patch removal, persisted at the end of the observation period (7d).</td><td>Thyssen, J. (1982) (CA) B.6.2.4-02</td></tr>
<tr><td>Skin irritation/ corrosion in rabbits
OECD TG 404
GLP: No
Deviations: Exposure time was 30 min instead of 4 h.
Reporting deficits: test material not characterised, individual body weights not reported.
Supportive only</td><td>Species: rabbit
Strain: New Zealand White
3 male rabbits</td><td>ortho-Phenylphenol (OPP)
Purity: not indicated
Dose: 0.5 g

30 min. exposure</td><td>Results:
<table><tr><th rowspan="2">Observation time</th><th colspan="6">Rabbit No.</th></tr><tr><th colspan="2">100</th><th colspan="2">107</th><th colspan="2">108</th></tr><tr><th>E*</th><th>O*</th><th>E</th><th>O</th><th>E</th><th>O</th></tr><tr><td>1 h</td><td>2</td><td>1</td><td>1</td><td>1</td><td>2</td><td>1</td></tr><tr><td>24 h</td><td>2</td><td>0</td><td>1</td><td>0</td><td>1</td><td>0</td></tr><tr><td>48 h</td><td>1</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td></tr><tr><td>72 h</td><td>1</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td></tr><tr><td>10 d</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr><tr><td>Mean 24/48/72 h</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>* E: erythema, O: oedema

Only 30 min. of exposure period. All signs were recovered after the 10-day observation period.</td><td>Suberg, H. (1983) (CA) B.6.2.4-03</td></tr>
<tr><td>Skin irritation/ corrosion in rabbits
Prior to OECD TG 404
GLP: No
Deviations:</td><td>Species: rabbit
Strain: New Zealand White
1 rabbit/sex</td><td>ortho-Phenylphenol (OPP)
Purity: not indicated
Dose: 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.</td><td>Thyssen, J. (1978) (CA) B.6.2.4-04</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group,</th><th>Test substance, dose levels, duration of exposure</th><th>Results
- Observations and time point of onset
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr><td>One page report in German. Test material not characterised; application onto the inner surface of the ear; exposure time 24 h instead of 4 h; skin irritation scores and individual body weights were not reported.
Supportive only</td><td></td><td>24 h exposure</td><td></td><td></td></tr>
<tr><td>Skin irritation/ corrosion in rabbits and humans
Prior to OECD TG 404
GLP: No
Deviations: Brief summary in German, with no translation.
Test substances 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: 24 h. Skin irritation scores not reported.
2 rabbits and 11 human volunteers per test substance.
Supportive only</td><td>Species: rabbit and human
Strain: not specified
2 rabbits/group
11 human subjects/group</td><td>o-Oxydiphenyl (OPP) and m-Oxydiphenyl

Dose: 0.1% aqueous solutions

24 h 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.</td><td>Kimmerle, G. and Lorke, D. (1969) (CA) B.6.2.4-05</td></tr>
<tr><td>Skin irritation/ corrosion in rabbits

OECD TG 404
GLP: No</td><td>Species: rabbit
Strain: New Zealand White
3 male rabbits</td><td>ortho-Phenylphenol (OPP).
Purity: 99.5%
Dose: 0.5 g</td><td>Results:
<table>
<tr><th rowspan="2">Observation time</th><th colspan="6">Rabbit No.</th></tr>
<tr><th colspan="2">1</th><th colspan="2">2</th><th colspan="2">3</th></tr>
<tr><th>E*</th><th>O*</th><th>E</th><th>O</th><th>E</th><th>O</th></tr>
<tr><td>1 h</td><td>1</td><td>2</td><td>0</td><td>0</td><td>1</td><td>1</td></tr>
<tr><td>24 h</td><td>1</td><td>1</td><td>2</td><td>0</td><td>1</td><td>0</td></tr>
</table></td><td>Schreiber, G. (1981a) (CA) B.6.2.4-06</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group,</th><th>Test substance, dose levels, duration of exposure</th><th>Results
- Observations and time point of onset
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr><td>Study acceptable</td><td></td><td>Applied formulated as a paste

4 h exposure</td><td>
<table>
<tr><td>48 h
72 h</td><td>1
1</td><td>1
1</td><td>2
2</td><td>0
0</td><td>1
1</td><td>0
0</td></tr>
<tr><td>8 d</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Mean 24/48/72 h</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>
* E: erythema, O: oedema
All the signs of irritation were reversible at the end of the observation period (8 days).</td><td></td></tr>
<tr><td>Skin irritation/ corrosion in rabbits

OECD TG 404
GLP: Yes

Study acceptable</td><td>Species: rabbit
Strain: New Zealand White
3 male rabbits</td><td>Sodium ortho-phenylphenate (SOPP)
Purity: 76.2/76.4%
Dose: 0.5 g Applied as a paste

4 h exposure</td><td>Results:
<table>
<tr><th rowspan="2">Observation time</th><th colspan="6">Rabbit No.</th></tr>
<tr><th colspan="2">A33</th><th colspan="2">A25</th><th colspan="2">A64</th></tr>
<tr><th>E*</th><th>O*</th><th>E</th><th>O</th><th>E</th><th>O</th></tr>
<tr><td>1 h</td><td>3</td><td>3</td><td>3</td><td>3</td><td>3</td><td>3</td></tr>
<tr><td>24 h</td><td>4**</td><td>4**</td><td>4**</td><td>4**</td><td>4**</td><td>4**</td></tr>
<tr><td>48 h</td><td>4**</td><td>4**</td><td>4**</td><td>4**</td><td>4**</td><td>4**</td></tr>
<tr><td>72 h</td><td>4**</td><td>4**</td><td>4**</td><td>4**</td><td>4**</td><td>4**</td></tr>
<tr><td>8 d</td><td>4**</td><td>4**</td><td>4**</td><td>4**</td><td>4**</td><td>4**</td></tr>
<tr><td>Mean 24/48/72 h</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td></tr>
<tr><td>Reversible</td><td>N</td><td>N</td><td>N</td><td>N</td><td>N</td><td>N</td></tr>
</table>
* E: erythema, O: oedema
**Necrotic changes observed
All 3 animals showed oedema and erythema scores of 3, at the 1-h reading, which resulted in oedema and erythema scores of 4 at 24-, 48- and 72-h scoring.
In addition, the skin of all 3 rabbits displayed necrotic changes from the 24-hour evaluation until the end of the study (day 8).</td><td>Märtins, T. (1988) (CA) B.6.2.4-07</td></tr>
<tr><td>Skin irritation/ corrosion in rabbits
Prior to OECD TG 404
GLP: No
Deviations: Test material not characterised. 24 h exposure; Readings only at the removal of the dressing, and 48 h and 7 d after. Duration of the 7 days (no reversibility clarified)
Supportive only</td><td>Species: rabbit
Strain: New Zealand White
1 male and 1 female rabbits</td><td>Sodium ortho-phenylphenate (SOPP)
Purity: not indicated

Dose: 0.5 g

24 h exposure</td><td>Results:
<table>
<tr><th rowspan="2">Observation time</th><th colspan="4">Rabbit No.</th></tr>
<tr><th colspan="2">A33</th><th colspan="2">A64</th></tr>
<tr><th>E*</th><th>O*</th><th>E</th><th>O</th></tr>
<tr><td>1 h</td><td>4</td><td>3</td><td>4</td><td>3</td></tr>
<tr><td>24 h</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>48 h</td><td>4</td><td>3</td><td>4</td><td>3</td></tr>
<tr><td>72 h</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>8 d</td><td>4</td><td>2</td><td>4</td><td>2</td></tr>
<tr><td>Mean 24/48/72 h</td><td>n/a</td><td>n/a</td><td>n/a</td><td>n/a</td></tr>
<tr><td>Reversible</td><td>N</td><td>N</td><td>N</td><td>N</td></tr>
</table>
* E: erythema, O: oedema
Reddening of the skin, grade 4, was observed since the patch removal until the last skin scoring (7 days). This grade 4 was described in the report as “deep reddening, partially burn”.
Swelling of the skin with a score of 3 was determined in both rabbits at the 0-h and 24-h reading time points, which slightly decreased to a score of 2 at the end of the observation period (7 d).</td><td>Pauluhn, J. (1983) (CA) B.6.2.4-08</td></tr>
</table>

Table 28: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance	Relevant	Observations	Reference

		information about the study (as applicable)		
No data available.				

Table 29: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Acute dermal toxicity study in rats *(see point 2.6.2.2 for more details)*	*ortho*-phenylphenol (**OPP**) Purity: 99.89%	Wistar rats 5/sex Dermal Single dose Dose: 2000 mg/kg bw Vehicle: Cremophor E 24-h exposure	Clinical signs: Slight reddening of 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.	Bomhard, E. (1991) (CA) B.6.2.2-01
Acute dermal toxicity study in rabbits (see point 2.6.2.2 for more details)	*ortho*-phenylphenol (**OPP**) Purity: 99.73%	New Zealand White rabbits 2/sex Dermal Single dose Dose: 5000 mg/kg bw applied dry on the skin. Water added to simulate moistened skin. 24-h exposure	Topical responses observed on the application sites of test rabbits 24 hours post-treatment included slight to moderate erythema, moderate oedema, and marked necrosis at the application site in all treated rabbits.	Carreon, R.E. and New, M.A. (1981) (CA) B.6.2.2-02
Acute dermal toxicity study in rats (see point 2.6.2.2 for more details)	Sodium ortho-phenylphenate (SOPP) Purity: not indicated	Wistar rats 5/sex Dermal Single dose Dose: 2000 mg/kg bw 24-h exposure	Local effects: All animals died during the first 5 days of the study: one was found dead on day 5 and the others were sacrified for humane reasons after considering the severity of the necrosis produced by the substance. The death of the animal that died was also considered related to necrosis.	Busschers, M (1997) (CA) B.6.2.2-03
Dermal 21-day study, rat *(See point 2.6.3.1 for more details)*	*ortho*-Phenylphenol (**OPP**) Purity: 99.82%	Fischer 344 rats 5 rats/sex/dose Repeated dermal application 6 h exposure, 5days/week for 21 days Doses: 0, 100, 500 or 1000 mg/kg bw	Local effects: Hyperkeratosis and acanthosis indicative of the OPP-induced irritation were found in 1/5 male and 4/5 females treated at 500 mg/kg and in 3/5 males and 4/5 females treated at 1000 mg/kg.	Zempel, J.A. and Szabo, J.R. (1993) (CA) B.6.3.3-01
Dermal 4-week study, mice	*ortho*-Phenylphenol	Swiss Webster CF	Local effects: Ulcerative lesions at the site of application were observed	National Toxicology

<table>
<tr><th>Type of study/data</th><th>Test substance</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td>(Range finding study for the carrcionogenicity dermal study B.6.5-05)

(See point 2.6.3.1 for more details)</td><td>(OPP)
Purity: >99%</td><td>W mice 10/sex/dose
Repeated dermal application 3days/week for 4 weeks
Doses: 0, 5.95, 11.4, 20.8, 35.7, or 55.5 mg per animal</td><td>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.</td><td>Program (1986) (CA) B.6.3.3-02</td></tr>
<tr><td>Long-term dermal, mouse.

(See point 2.5 for more details)</td><td>OPP purity >99%)</td><td>Swiss CD-1 mice 50/sex and dose.
Repeated dermal application 0, 55.5 mg OPP/animal/ day, 3 days a week, (with or without 0.05 mg of DMBA pre-treatment)for 102 weeks.
An additional positive control group was treated with:
0.05 mg DMBA, then 0.005 mg of TPA</td><td>▪ Skin: Non-neoplastic lesions in ♂ and ♀ (ulcers, active chronic inflammation, hyperkeratosis, and acanthosis) at the site of application in all groups, with an increased incidence in male and female mice of the OPP, DMBA/OPP, or DMBA/TPA treatment groups (see table below).
Incidence of skin lesions at the application site
<table>
<tr><th rowspan="2">Lesion</th><th colspan="2">Acetone</th><th colspan="2">OPP</th><th colspan="2">DMBA</th><th colspan="2">DMBA/ OPP</th><th colspan="2">DMBA/ TPA</th></tr>
<tr><th>M</th><th>F</th><th>M</th><th>F</th><th>M</th><th>F</th><th>M</th><th>F</th><th>M</th><th>F</th></tr>
<tr><td>Ulcer</td><td>5</td><td>1</td><td>19</td><td>11</td><td>2</td><td>7</td><td>16</td><td>11</td><td>15</td><td>12</td></tr>
<tr><td>Active chronic inflammation</td><td>10</td><td>7</td><td>25</td><td>20</td><td>10</td><td>7</td><td>25</td><td>27</td><td>27</td><td>25</td></tr>
<tr><td>Hyperkeratosis</td><td>7</td><td>4</td><td>27</td><td>16</td><td>8</td><td>4</td><td>24</td><td>27</td><td>30</td><td>26</td></tr>
<tr><td>Acanthosis</td><td>13</td><td>4</td><td>44</td><td>36</td><td>12</td><td>12</td><td>33</td><td>42</td><td>44</td><td>41</td></tr>
<tr><td>Squamous cell papilloma</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>4</td><td>4</td><td>2</td><td>7</td><td>17</td></tr>
<tr><td>Squamous cell carcinoma</td><td>0</td><td>0</td><td>0</td><td>0</td><td>4</td><td>3</td><td>1</td><td>3</td><td>18</td><td>18</td></tr>
<tr><td>Basal cell tumour</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>2</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>Basal cell carcinoma</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2</td><td>2</td><td>3</td><td>0</td><td>2</td></tr>
<tr><td>Keratoacanthoma</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>5</td></tr>
<tr><td>Sebaceous adenoma</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>1</td><td>1</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Sebaceous adenocarcinoma</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Neoplastic skin lesion (combined)</td><td>0</td><td>0</td><td>0</td><td>0</td><td>6</td><td>9</td><td>9</td><td>8</td><td>19</td><td>32</td></tr>
</table></td><td>Toxicology Program, (1986) (CA) B.6.5-05</td></tr>
</table>

2.6.2.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

All the available skin corrosion/irritation studies for ***ortho*-phenylphenol (OPP)** were included and assessed in the previous DAR (2008).

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 under GLP (B.6.2.4-01). These two studies show conflicting results: in the first one (Gilbert, K.S., 1994a; B.6.2.4-01), severe skin effects were observed in 4 of 6 animals, with the formation of scars (which are evidence of corrosion). This finding was described as "burns observed at application site" in the study summary of the previous DAR (2008), and a classification as R38 (irritating to skin) was proposed in accordance with EU Commission Directive 2001/59/EC. The study has been reassessed for this review, and the transformation of this burnings into scars was confirmed in the study report.

In the contrary, the other acceptable study (Schreiber, G., 1981a; 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 that explains such a difference between the two studies is not clear. The first one is more recent (1994), it was conducted under GLP, used 6 rabbits and the test substance was applied moistened with 0.3 ml of distilled water; after 4 hour exposure in a Hill Top Chamber, the residual substance was wiped with a damp disposable towel. The second study is older (1981), when GLP were not mandatory, 3 rabbits were used and the test substance was formulated as a paste in water (proportion not specified), applied for 4 hours in semi-occlusive conditions, and the residual substance was removed with water or olive oil. These are the main differences observed in the procedures, but they are not considered strong enough to explain the conflicting results obtained in the two studies. Furthermore, it should be noted that skin responses of two of the rabbits of the first study (slight erythema which was reversible in 48 hours) were more similar to the ones observed in the second study, despite the abovementioned differences in procedures.

Another 4 studies (B.6.2.4-02 to B.6.2.4-05) were provided for the assessment of the skin corrosion/irritation of OPP. These studies were considered as supportive due to methodological deficiencies, and therefore not acceptable for classification purposes.

Two of these studies did not even report irritation scores, and the exposure period lasted for 24 hours, so this data must be taken very carefully: in the first one (Thyssen, J., 1978; B.6.2.4-04), the substance showed to be moderately irritating to the skin, and the other study (Kimmerle, G. and Lorke, D., 1969; B.6.2.4-05) showed no irritation effects (an aqueous solution was used). This data cannot be used for the assessment, but to confirm the equivocal presence of both findings (irritantion and no effects) when the substance is applied on skin.

However, information can be drawn from the other two supplementary studies, which did report individual scores. One of these studies (Suberg, H., 1983; 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, which might be enough to determine a category 1B corrosion effect, but is not enough to contrast the established skin irritation/corrosion classification criteria. As for the last available study (Thyssen, J., 1982; B.6.2.4-02), animals were exposed for 4 hours, what can be comparable with the main studies (the acceptable ones: Gilbert, K.S., 1994a (B.6.2.4-01) and Schreiber, G., 1981a (B.6.2.4-06)). In this study, 5 rabbits showed moderate to severe erythema at the 72 h 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 4 animals 72 hours after exposure, which persisted until day 7. It should be noted that in this study, although 5 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.

Additional information can be found in other dermal toxicity studies, for a complete Weight-of-Evidence analysis, although the use of this data needs to be evaluated on a case-bycase basis (as mentioned in the Guidance on the Application of the CLP Criteria, 2017), due to the different protocols and the interspecies differences in sensitivity.

Two acute dermal toxicity studies are available: one was carried out with rats (Bomhard, E., 1991; B.6.2.2-01), where a slight reddening was observed in the application site on day 1, turned to incrustation on day 5, and was reversible by day 14. The other study (Carreon, R.E. and New, M.A., 1981; B.6.2.2-02) was carried out with 4 rabbits, and necrosis was observed at the application site in all the treated animals, which, again, is an evidence of corrosion.

Also, to support the evidence that the substance causes irritantion 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 (Zempel, J.A. and Szabo, J.R., 1993), and ulcerative lesions at the site of application in the other (National Toxicology Program, 1986).

Summarising the abovementioned information, two of the provided studies are considered relevant but show controversy in their results. While clear corrosive effects are shown in the most recent study, the irritation effects observed in the other study were reversible after 10 days. Another study, which used a 4-hour exposure period, is also available, although it was considered supportive (due to methodological deficits). This study resulted in severe skin lesions, which were not reversible at the end of the study (only 7 days) in five animals and slight effects in one. Moreover, an acute dermal toxicity study in rabbits, shows necrosis in the 4 animals tested.

Regarding the studies performed in other species (e.g. rat), the abovementioned guidance indicates that "*Considering the fact that (i) the rat skin is less sensitive compared to rabbit skin, (ii) much lower exposures are employed and (iii), in general, the scoring of dermal effects is performed less accurately, the results of dermal toxicity testing in rats will not be adequate for classification with respect to skin irritation. Only in case of evidence of skin corrosivity in the rat dermal toxicity test can the test substance be classified as Skin Corrosive Category 1. All other data should be used in a Weight of Evidence."* In this case, severe effects (including ulcers) were observed in rat and mouse, which are species less sensitive than rabbit. Classification for skin corrosion may not be justified based on these studies, but RMS is of the opinion that these data support the evidence observed in the first study (Gilbert, K.S., 1994a; B.6.2.4-01). No clear conclusion on the reason why one study would lead to the classification of OPP as skin corrosion, category 1 (H314), and the results of the other study do not fulfill the classification criteria for this hazard class. However, it should be noted that conflicting results can also be seen between different animals within the same study: while several animals show severe skin lesions, other animals, under the same conditions, present slight and/or reversible irritant effects; this effect could be seen in the two studies which showed severe lesions (Gilbert, K.S., 1994a; B.6.2.4-01 and Thyssen, J., 1982; B.6.2.4-02).

As a conclusion, it is considered that the first study (Gilbert, K.S., 1994a; B.6.2.4-01) is the most relevant to assess the potential of skin corrosion/irritation of *ortho*-phenylphenol, since in this study both effects (severe vs slight) where seen under the same conditions, and the severity of the skin reactions observed in 4 of 6 animals, with the presence of scars at the end of the 14-day observation period, should not be overlooked.

ortho-Phenylphenol classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). Classification regarding skin irritation is included as: Skin irritation, category 2 (Skin Irrit. 2; H315).

Regarding the data available on **sodium *ortho*-phenylphenate (SOPP)**, two new studies (Märtins, T., 1988; B.6.2.4-07 and Pauluhn, J., 1983; B.6.2.4-08) have been included for the renewal assessment of the active substance. No studies on skin corrosion/irritation of SOPP were included in the previous DAR (2008).

The study of Märtins, T. (1988; B.6.2.4-07) is GLP and guidance compliant and is considered acceptable to assess the classification of SOPP for this hazard class. The results of this study show necrotic changes in the three rabbits 24 hours after the patch removal. This lesion is considered an effect of the corrosive properties of the test substance.

The other available study on skin irritation/corrosion with SOPP (Pauluhn, J., 1983; B.6.2.4-08) is considered supportive only (due to methodological deficiencies), and also shows severe skin lesions (score 4, which is the maximum grade for erythema) that were persistent until the end of the study (8 days). Although the study report concludes that SOPP is a severe irritant (no corrosive), the description of the grade 4 erythema (deep reddening, partially burn) could be interpreted in both ways: as a severe irritation effect (deep reddening) or as a corrosive effect (burn). In conclusion, the outcome of this study supports the classification for the severe effects seen in the main study (Märtins, T., 1988; B.6.2.4-07)

Also, to support the evidence that the substance causes skin corrosion, the study provided for the evaluation of the acute dermal toxicity of SOPP (Busschers, M., 1997; B.6.2.2-03) should be considered, since the severe necrosis produced by SOPP derived in the death of one animal and the sacrifice for human reasons of the other 9 animals of the study.

Sodium *ortho*-phenylphenate classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). Classification regarding skin irritation is included as: Skin irritation, category 2 (Skin Irrit. 2; H315).

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

***ortho*-Phenylphenol (OPP):** According to the study of Gilbert, K.S. (1994a; B.6.2.4-01; guideline and GLP compliant study), after a exposure of 4 hours, 4 out of 6 rabbits showed a mean score per animal of 4.0 for erythema (which would lead to a classification as skin irritant, as concluded in DAR 2008, and proposed by the applicant). However, once reviewed the study, also scar formation was identified in these 4 animals at the end of the study (14 days observation period).

According to the current EU Criteria (Regulation 1272/2008), classification as skin corrosive is required if at least one animal shows a corrosive response (such as scars) at the end of the observation period. When data are sufficient substances shall be classified in one of the three sub-categories 1A, 1B, or 1C; otherwise, corrosive substances shall be classified in Category 1.

Moreover, according to the Guidance on the Application of the CLP Criteria (2017): *"If the substance is proven to be either an irritant or a corrosive in an acute dermal toxicity test carried out with rabbits with the undiluted test substance (liquids) or with a suitable suspension (solids), the following applies. In case of signs of skin corrosion, classify as Skin Corrosive (subcategorisation as 1A, 1B or 1C, where possible). […]".* In this case, an acute dermal toxicity test in rabbits is available, where OPP was applied dry (water was added to simulate moistened skin) and necrosis was observed in all the 4 rabbits treated.

According to the available data, RMS considers OPP fulfils the criteria to classify as category 1 (since only data from 4 hour exposure duration, no subcategorization can be concluded).

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Based on the data available for ***ortho*-phenylphenol (OPP)**, and according to the criteria under Regulation (EC) No. 1272/2008, RMS proposes the classification of this active substance as **skin corrosive, category 1, Skin Corr. 1 (H314)**.

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

	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

B.6.2.4-01, 1994

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

	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

B.6.2.4-02, 1982

		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.

Skin irritation/ corrosion in rabbits OECD TG 404 GLP: No NZW rabbits 3 males Supportive study	Purity: not indicated 0.5 g 30 min exposure		Rabbit No						B.6.2.4-03, 1983
			100		107		108		
			E	O	E	O	E	O	
		1h	2	1	1	1	2	1	
		24h	2	0	1	0	1	0	
		48h	1	0	0	0	1	0	
		72h	1	0	0	0	1	0	
		10d	0	0	0	0	0	0	
		Mean 24/48/72h	1.3	0.0	0.3	0.0	1.0	0.0	
		Reversible	Y	Y	Y	Y	Y	Y	
		Deviations: exposure time 30 min (instead of 4h); reporting deficits: test material not characterised, individual body weights not reported.							
Skin irritation/ corrosion in rabbits Prior to OECD TG 404 GLP: No NZW rabbit 1 rabbit/sex Supportive study	Purity: not indicated 0.5 g	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.							B.6.2.4-04, 1978
Skin irritation/ corrosion in rabbits and humans Prior to OECD TG 404 GLP: No 2 rabbits and 11 human volunteers	0.1% aqueous OPP solution 24h exposure Application: - inner side of the ear of the rabbits and - lower arm of human subjects	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.							B.6.2.4-05, 1969

Skin irritation/ corrosion in rabbits OECD TG 404 GLP: No NZW rabbits 3 males Key study	Purity: 99.5% 0.5 g		Rabbit No					B.6.2.4-06, 1981a
			1		2		3	
			E	O	E	O	E	O
		1h	1	2	0	0	1	1
		24h	1	1	2	0	1	0
		48h	1	1	2	0	1	0
		72h	1	1	2	0	1	0
		8d	0	0	0	0	0	0
		Mean 24/48/72h	1.0	1.0	2.0	0.0	1.0	0.0
		Reversible	Y	Y	Y	Y	Y	Y

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**.

2.6.2.5 Serious eye damage/eye irritation [equivalent to section 10.5 of the CLH report template]

Table 30: Summary table of animal studies on serious eye damage/eye irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance, Dose levels, duration of exposure</th><th>Results
- Observations and time point of onset
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr>
<td>Eye irritation, OECD TG 405, GLP: No

Deviations: Observation period of 8 days instead of 21.

Study acceptable</td>
<td>Rabbit, New Zealand White, 3 males</td>
<td>ortho-Phenylphenol (OPP)
Purity: 99.5%
100 µl,
no rinsing</td>
<td>Results:
<table>
<tr><th rowspan="3">Obs. time</th><th colspan="4">Rabbit 1</th><th colspan="4">Rabbit 2</th><th colspan="4">Rabbit 3</th></tr>
<tr><th rowspan="2">Cornea</th><th rowspan="2">Iris</th><th colspan="2">Conj.</th><th rowspan="2">Cornea</th><th rowspan="2">Iris</th><th colspan="2">Conj.</th><th rowspan="2">Cornea</th><th rowspan="2">Iris</th><th colspan="2">Conj.</th></tr>
<tr><th>E*</th><th>O*</th><th>E*</th><th>O*</th><th>E*</th><th>O*</th></tr>
<tr><td>1 h</td><td>1</td><td>0</td><td>1</td><td>1</td><td>1</td><td>0</td><td>1</td><td>2</td><td>2</td><td>0</td><td>1</td><td>2</td></tr>
<tr><td>24 h</td><td>1</td><td>0</td><td>1</td><td>3</td><td>1</td><td>0</td><td>2</td><td>3</td><td>2</td><td>0</td><td>1</td><td>2</td></tr>
<tr><td>48 h</td><td>2</td><td>2</td><td>2</td><td>2</td><td>2</td><td>1</td><td>2</td><td>2</td><td>2</td><td>1</td><td>1</td><td>2</td></tr>
<tr><td>72 h</td><td>2</td><td>2</td><td>2</td><td>2</td><td>2</td><td>1</td><td>2</td><td>2</td><td>2</td><td>1</td><td>2</td><td>2</td></tr>
<tr><td>8 d</td><td>2</td><td>2</td><td>1</td><td>1</td><td>3</td><td>2</td><td>1</td><td>1</td><td>2</td><td>2</td><td>1</td><td>1</td></tr>
<tr><td>Mean 24/48/72 h</td><td>1.67</td><td>1.33</td><td>1.67</td><td>2.33</td><td>1.67</td><td>0.67</td><td>2.0</td><td>2.33</td><td>2.0</td><td>0.67</td><td>1.33</td><td>2.0</td></tr>
<tr><td>Revers** (72 h - 8 d)</td><td>N</td><td>N</td><td>Y↓</td><td>Y↓</td><td>N↑</td><td>N↑</td><td>Y↓</td><td>Y↓</td><td>N</td><td>N↑</td><td>Y↓</td><td>Y↓</td></tr>
</table>
* E: erythema / O: oedema
** Evidence of reversibility between the 72 h and 7 days evaluations: N↑ (increased lesion score); N (same score); Y↓ (decreased score); Y (fully reversible)

The study was finalised after 8 days instead of 21, and the reversibility of the lesions could not be properly assessed. At the end of the study, scores for corneal opacity and iritis were not lower than the previous observation time (72h): the corneal opacity reached a grade 3 in one animal, and for two animals the severity of iritis also increased (from grade 1 after 72 h, to grade 2 after 8 days).</td>
<td>Schreiber, G., 1981b (CA) B.6.2.5-01</td>
</tr>
<tr>
<td>Eye irritation, No guidelines, GLP: No

Deviations: Purity and</td>
<td>Rabbit, New Zealand White, 1 male and 1 female</td>
<td>ortho-Phenylphenol (OPP)</td>
<td>The test article was strongly irritating and corrosive.
No data on ocular lesions scores nor any other data was reported.</td>
<td>Thyssen, J. (1978) (CA) B.6.2.5-02</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance, Dose levels, duration of exposure</th><th>Results
- Observations and time point of onset
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr><td>batch not reported.
No data of ocular lesions score neither any other data was reported
Supportive only</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Eye irritation, OECD TG 405,
GLP: No

Deviations: Observation period of 7 days instead of 21.
Supportive only</td><td>Rabbit, New Zealand White,
5 males and
1 female</td><td>ortho-Phenylphenol (OPP)
Lot. MM101071 1 Z

0.1 g</td><td>Results:
<table>
<tr><th rowspan="3">Obs. time</th><th colspan="4">Rabbit 1</th><th colspan="4">Rabbit 2</th><th colspan="4">Rabbit 3</th></tr>
<tr><th rowspan="2">Cornea</th><th rowspan="2">Iris</th><th colspan="2">Conj.</th><th rowspan="2">Cornea</th><th rowspan="2">Iris</th><th colspan="2">Conj.</th><th rowspan="2">Cornea</th><th rowspan="2">Iris</th><th colspan="2">Conj.</th></tr>
<tr><th>E*</th><th>O*</th><th>E*</th><th>O*</th><th>E*</th><th>O*</th><th>E*</th><th>O*</th></tr>
<tr><td>24 h</td><td>1</td><td>1</td><td>2</td><td>3</td><td>2</td><td>1</td><td>2</td><td>4</td><td>2</td><td>1</td><td>3</td><td>4</td></tr>
<tr><td>48 h</td><td>1</td><td>1</td><td>3</td><td>4</td><td>2</td><td>1</td><td>3</td><td>3</td><td>2</td><td>1</td><td>3</td><td>4</td></tr>
<tr><td>72 h</td><td>2</td><td>1</td><td>3</td><td>3</td><td>3</td><td>1</td><td>3</td><td>3</td><td>2</td><td>1</td><td>3</td><td>4</td></tr>
<tr><td>7 d</td><td>2</td><td>1</td><td>1</td><td>2</td><td>3</td><td>1</td><td>2</td><td>3</td><td>2</td><td>1</td><td>2</td><td>2</td></tr>
<tr><td>Mean 24/48/72 h</td><td>1.33</td><td>1</td><td>2.67</td><td>3.33</td><td>2.33</td><td>1</td><td>2.67</td><td>3.33</td><td>2</td><td>1</td><td>3</td><td>4</td></tr>
<tr><td>Revers** (72 h- 7 d)</td><td>N</td><td>N</td><td>Y↓</td><td>Y↓</td><td>N</td><td>N</td><td>Y↓</td><td>N</td><td>N</td><td>N</td><td>Y↓</td><td>Y↓</td></tr>
</table>
<table>
<tr><th rowspan="3">Obs. time</th><th colspan="4">Rabbit 4</th><th colspan="4">Rabbit 5</th><th colspan="4">Rabbit 6</th></tr>
<tr><th rowspan="2">Cornea</th><th rowspan="2">Iris</th><th colspan="2">Conj.</th><th rowspan="2">Cornea</th><th rowspan="2">Iris</th><th colspan="2">Conj.</th><th rowspan="2">Cornea</th><th rowspan="2">Iris</th><th colspan="2">Conj.</th></tr>
<tr><th>E*</th><th>O*</th><th>E*</th><th>O*</th><th>E*</th><th>O*</th><th>E*</th><th>O*</th></tr>
<tr><td>24 h</td><td>2</td><td>1</td><td>2</td><td>4</td><td>3</td><td>1</td><td>2</td><td>4</td><td>2</td><td>1</td><td>2</td><td>4</td></tr>
<tr><td>48 h</td><td>3</td><td>1</td><td>2</td><td>4</td><td>3</td><td>1</td><td>2</td><td>4</td><td>1</td><td>1</td><td>2</td><td>3</td></tr>
<tr><td>72 h</td><td>3</td><td>1</td><td>3</td><td>4</td><td>3</td><td>1</td><td>3</td><td>4</td><td>2</td><td>1</td><td>2</td><td>2</td></tr>
<tr><td>7 d</td><td>2</td><td>1</td><td>2</td><td>2</td><td>2</td><td>1</td><td>2</td><td>2</td><td>2</td><td>1</td><td>2</td><td>2</td></tr>
<tr><td>Mean 24/48/72 h</td><td>2.67</td><td>1</td><td>2.33</td><td>4</td><td>3</td><td>1</td><td>2.33</td><td>4</td><td>1.67</td><td>1</td><td>2</td><td>3</td></tr>
<tr><td>Revers** (72 h- 7 d)</td><td>Y↓</td><td>N</td><td>Y↓</td><td>Y↓</td><td>Y↓</td><td>N</td><td>Y↓</td><td>Y↓</td><td>N</td><td>N</td><td>N</td><td>N</td></tr>
</table>
* E: erythema / O: oedema
** Evidence of reversibility between the 72 h and 7 days evaluations: N↑ (increased lesion score); N (same score); Y↓ (decreased score); Y (fully reversible)
<u>Comments</u>: Slight to moderate pain after instillation.

The study was finalised after 7 days instead of 21, and the reversibility of the lesions could not be properly assessed. At the end of the study, the 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 with no evidence of reversibility after 7 days.</td><td>Norris, J.M., (1971) (CA) B.6.2.5-03</td></tr>
<tr><td>Eye irritation, OECD TG 405,
GLP: Yes

Study acceptable</td><td>Rabbit, New Zealand White,
3 males</td><td>Sodium ortho-phenylphenate (SOPP)

0.1 ml
Eye rinsed 24h post-instillation</td><td>Results:
<table>
<tr><th rowspan="3">Obs. time</th><th colspan="4">Rabbit No. A19</th><th colspan="4">Rabbit No. A77</th><th colspan="4">Rabbit No. A26</th></tr>
<tr><th rowspan="2">Cornea</th><th rowspan="2">Iris</th><th colspan="2">Conj.</th><th rowspan="2">Cornea</th><th rowspan="2">Iris</th><th colspan="2">Conj.</th><th rowspan="2">Cornea</th><th rowspan="2">Iris</th><th colspan="2">Conj.</th></tr>
<tr><th>E*</th><th>O*</th><th>E*</th><th>O*</th><th>E*</th><th>O*</th><th>E*</th><th>O*</th></tr>
<tr><td>1 h[1]</td><td>2</td><td>1</td><td>2</td><td>1</td><td>2</td><td>1</td><td>2</td><td>1</td><td>1</td><td>1</td><td>2</td><td>1</td></tr>
<tr><td>24 h</td><td>2</td><td>1</td><td>2</td><td>2</td><td>2</td><td>1</td><td>2</td><td>2</td><td>1</td><td>1</td><td>2</td><td>1</td></tr>
<tr><td>48 h</td><td>2</td><td>1</td><td>2</td><td>2</td><td>2</td><td>1</td><td>2</td><td>2</td><td>1</td><td>1</td><td>2</td><td>1</td></tr>
<tr><td>72 h</td><td>2</td><td>1</td><td>2</td><td>2</td><td>2</td><td>1</td><td>2</td><td>3</td><td>1</td><td>1</td><td>2</td><td>1</td></tr>
<tr><td>7 d[2]</td><td>3</td><td>1</td><td>2</td><td>1</td><td>3</td><td>1</td><td>2</td><td>2</td><td>1</td><td>1</td><td>2</td><td>1</td></tr>
<tr><td>Mean 24/48/72 h</td><td>2.0</td><td>1.0</td><td>2.0</td><td>2.0</td><td>2.0</td><td>1.0</td><td>2.0</td><td>2.3</td><td>1.0</td><td>1.0</td><td>2.0</td><td>1.0</td></tr>
</table></td><td>Märtins, T. (1988) (CA) B.6.2.5-04</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance, Dose levels, duration of exposure</th><th>Results
- Observations and time point of onset
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
<table>
<tr><td>Revers** (72 h- 7 d)</td><td>N↑</td><td>N</td><td>N</td><td>Y↓</td><td>N↑</td><td>N</td><td>N</td><td>Y↓</td><td>N</td><td>N</td><td>N</td><td>N</td></tr>
</table>
* E: erythema / O: oedema
** Evidence of reversibility between the 72 h and 7 days evaluations: N↑ (increased lesion score); N (same score); Y↓ (decreased score); Y (fully reversible)

Other findings:
[1] The mucous membrane of the third eyelid of the animals was necrotized from the first evaluation point on (1 h).
[2]At the 7-d evaluation point, Rabbit A19 showed a corneal pannus, and rabbit A77 hair loss at the upper and lower margin of the eyelid.
</td><td></td></tr>
<tr><td>Eye irritation, Prior to OECD TG 405,
GLP: No

Deviations: Test material not characterised

Supportive only</td><td>Rabbit, New Zealand White, 1/sex</td><td>Sodium ortho-phenylphenate (SOPP)
Purity: not indicated

0.1 ml</td><td>Results:
<table>
<tr><th rowspan="2">Obs. time</th><th colspan="4">Rabbit No. 235</th><th colspan="4">Rabbit No. 236</th></tr>
<tr><th>Cornea</th><th>Iris</th><th>Conj.</th><th></th><th>Cornea</th><th>Iris</th><th>Conj.</th><th></th></tr>
<tr><th></th><th></th><th></th><th>E*</th><th>O*</th><th></th><th></th><th>E*</th><th>O*</th></tr>
<tr><td>1 h</td><td>1</td><td>1</td><td>3</td><td>2</td><td>1</td><td>0</td><td>3</td><td>3</td></tr>
<tr><td>24 h</td><td>1</td><td>1</td><td>3</td><td>2</td><td>2</td><td>2</td><td>3</td><td>2</td></tr>
<tr><td>48 h</td><td>1</td><td>1</td><td>3</td><td>2</td><td>2</td><td>2</td><td>3</td><td>2</td></tr>
<tr><td>72 h</td><td>1</td><td>1</td><td>3</td><td>2</td><td>2</td><td>2</td><td>3</td><td>2</td></tr>
<tr><td>8 d</td><td>1</td><td>0</td><td>1</td><td>1</td><td>2</td><td>1</td><td>2</td><td>2</td></tr>
<tr><td>Mean 24/48/72 h</td><td>1.0</td><td>1.0</td><td>3.0</td><td>2.0</td><td>2.0</td><td>2.0</td><td>3.0</td><td>2.0</td></tr>
<tr><td>Revers** (72 h- 8 d)</td><td>N</td><td>Y</td><td>Y↓</td><td>Y↓</td><td>N</td><td>Y↓</td><td>Y↓</td><td>N</td></tr>
</table>
* E: erythema / O: oedema
** Evidence of reversibility between the 72 h and 7 days evaluations: N↑ (increased lesion score); N (same score); Y↓ (decreased score); Y (fully reversible)
Only effects in the iris of one animal were fully reversible at the 7-day reading time point.</td><td>Pauluhn, J. (1983) (CA) B.6.2.5-05</td></tr>
</table>

Table 31: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 32: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

2.6.2.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

All the available studies on serious eye damage/eye irritation for ***ortho*-phenylphenol (OPP)** were included and assessed in the previous DAR (2008). Only one of these three studies (Schreiber, G., 1981b; B.6.2.5-01) is considered acceptable (compliant with the guidance test methods), although the observation period was too short

to verify the reversibility of the lesions observed.

A second study is available (Norris, J.M., 1971; B.6.2.5-03), and considered as supplementary due to methodological deficits (previous to test methods guidelines). However, this study shows individual scorings that are consistent with the results observed in the first study, and is considered relevant to support the conclusions of the assessment.

As for the last study (Thyssen, J., 1978, B.6.2.5-02), only a document of one page in German is available, and is considered supplementary only, since only a statement was provided as a result (eye irritation scores not reported), that supports the corrosive findings evidenced in the previous studies: "The test article was strongly irritating and corrosive".

As a conclusion on the relevance of the available studies, although the three of them show serious effects, only the results obtained in the (Schreiber, G., 1981b; B.6.2.5-01) are considered acceptable for classification purposes of this hazard, and only the study (Norris, J.M., 1971; B.6.2.5-03) is considered supportive for the evaluation.

Ortho-Phenylphenol classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). Classification regarding eye damage/eye irritation is included as: Eye irritation, category 2 (Eye Irrit. 2; H319).

Regarding the data available on **sodium *ortho*-phenylphenate (SOPP)**, two new studies (Märtins, T., 1988; B.6.2.5-04 and Pauluhn, J., 1983; B.6.2.5-05) have been included for the renewal assessment of the active substance. No studies on skin corrosion/irritation of SOPP were included in the previous DAR (2008).

The study of Märtins, T. (1988; B.6.2.5-04) is GLP and guidance compliant and is considered acceptable to assess the classification of SOPP for this hazard class. After the administration of SOPP in the eye of three rabbits, corneal opacity scores grade 2 (from the 1 h to the 72 h readings) of two rabbits increased to grade 3 on day 7, with the formation of a corneal pannus in one of the animals (day 7). Necrosis of the nictitating membrane was also observed after the first observation time point in the three rabbits.

The other available study on skin irritation/corrosion with SOPP (Pauluhn, J., 1983; B.6.2.5-05) is considered supportive only (due to methodological deficiencies), and also shows severe eye lesions: the study was finalised after 7 days, when only effects in the iris of one animal were fully reversible, and considering the substance as caustic to the eye. In conclusion, the outcome of this study supports the classification for the severe effects seen in the main study (Märtins, T., 1988; B.6.2.5-04)

Sodium *ortho*-phenylphenate classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). Classification regarding eye damage/eye irritation is included as: Eye damage, category 1 (Eye Dam. 1; H318).

2.6.2.5.2 Comparison with the CLP criteria regarding serious eye damage/eye irritation

***Ortho*-Phenylphenol (OPP):** Considering OPP is proposed to be classified as skin corrosive (Skin Corr. 1; H314), classification as as serious eye damage, category 1 (Eye Dam.1; H318) is also required. Since the hazard statement H318 is already included in the hazard statement H314 for skin corrosion (Causes severe skin burns and eye damage), H318 is considered in this section, but it is not included in the End Points for labelling purposes to avoid redundancy (according to the Guidance on the Application of CLP Criteria, July 2017).

This conclusion is supported by the available data: The study of Schreiber, G., 1981b (B.6.2.5-01) is considered relevant to evaluate serious eye damage/eye irritation. However, due to the deviations observed (observation period of 8 days instead of 21), reversibility of the lesions could not be demonstrated.

As indicated by the applicant, the calculated mean scores following grading at 24, 48 and 72 hours after instillation of the test material, for the three rabbits, fulfil the criteria for classification as eye irritant (Eye Irrit. 2): corneal opacity ($\geq$ 1) and conjunctival oedema ($\geq$ 2). Furthermore, the mean scores for corneal (<3) and iris lesions ($\leq$ 1.5) are not high enough to fulfil the 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 which are not fully reversible within an observation period of normally 21 days). In this case, the study was finalised after 8 days. 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 also increased (from grade 1 after 72 h, 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 24/48/72 h) for classification of substances as Category 1.

Similar results are observed in the study of Norris, J.M., 1971 (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 72 h after the instillation.

As indicated by the applicant for this study, 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 (Eye Irrit. 2), as proposed by applicant: corneal opacity (≥ 1), iritis (≥ 1), and conjunctival redness (≥ 2) and oedema (≥ 2). Furthermore, the mean scores for corneal and iris lesions are not high enough to fulfil the criteria for classification as serious eye damage (Eye Dam. 1). 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.

Although this second study is considered as supportive only (due to methodological deviations), the severity of the results are consistent with the Schreiber, G., 1981b (B.6.2.5-01) study, and no clear evidence of reversibility can be seen in this study either.

During the evaluation of the previous DAR (2008), the same approach to this point was discussed in the Expert Meeting 59 (13-17 October 2008), ending with this conclusion: “the findings were sufficiently severe to propose R41 (“Risk of serious damage to eyes”). It was noted the ECB did not classify it as R41”.

Considering both the proposed classification of OPP as Skin Corr. 1 (H314) and the severity of the remaining iris and corneal lesions, showing no reversibility after 8 days (end of the study), RMS proposes classification of *ortho*-phenylphenol as serious eye damage, category 1 (Eye Dam.1; H318).

2.6.2.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Based on the data available for ***ortho*-phenylphenol (OPP)**, and according to the criteria under Regulation (EC) No. 1272/2008, RMS proposes the classification of this active substance as **serious eye damage, category 1, Eye Dam. 1 (H318).**

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>
</td><td>B.6.2.5-01, 1981b</td></tr>
</table>

	Rabbit 3			
			Conjunctiva	
	C	I	E	O
1h	2	0	1	2
24h	2	0	1	2
48h	2	1	1	2
72h	2	1	2	2
8d	2	2	1	1
Mean 24/48/72h	2.0	0.67	1.33	2.0
Revers (72h – 8d)	N	N↑	Y↓	Y↓

N↑ = increased lesion score; N = same score; Y↓ = decreased score

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	Dose not known	The test article was strongly irritating and corrosive. No data on ocular lesions scores nor any other data was reported.	B.6.2.5-02, 1978

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**.

2.6.2.6 Respiratory sensitisation [equivalent to section 10.6 of the CLH report template]

Table 33: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
No data available.					

Table 34: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 35: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

2.6.2.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

No data available, neither for *ortho*-phenylphenol (OPP) nor for sodium *ortho*-phenylphenate (SOPP).

2.6.2.6.2 Comparison with the CLP criteria regarding respiratory sensitisation

No data available.

2.6.2.6.3 Conclusion on classification and labelling for respiratory sensitisation

In the absence of any data, no classification for respiratory sensitisation can be drawn for *ortho*-phenylphenol (OPP).

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.

2.6.2.7 Skin sensitisation [equivalent to section 10.7 of the CLH report template]

Table 36: Summary table of animal studies on skin sensitisation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance, Dose levels, duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Skin sensitization in guinea pig, US EPA 81-6 (Buehler method), comparable to OECD 406 (1992)
GLP: Yes

Deviations: Only 10 animals treated. No negative control group. Test substance in a solid flake form.
Supportive only</td><td>Guinea Pig Hartley, males

Test group: 10
Positive control group: 10
No negative control group</td><td>ortho-Phenylphenol (OPP)
0.4 g (100% solid OPP) for induction and challenge phases

Poitive: DER 331 espoxi resin: 10% for induction and 7.5 for challenge</td><td>Results:
<table><tr><th>Time</th><th>Test compound group</th><th>Positive control group</th></tr><tr><td>24 h</td><td>0/10</td><td>8/10</td></tr><tr><td>48 h</td><td>0/9*</td><td>9/10</td></tr></table>*1 non-treatment-related death in treated group.</td><td>Berdasco, N.M. (1991) (CA) B.6.2.6-01</td></tr>
<tr><td>Skin sensitization in guinea pig, OECD 406 (1987) - Buehler method
GLP: Yes

Deviations: Only 10 animals in the treated group (required: ≥ 20 animals).
Only 5 animals in the control group (required: ≥ 10 animals). No justification was given for the use of a naive control group instead of a</td><td>Guinea Pig Hartley, males

Test group: 10
Positive control group: 10
Negative control groups (OPP and DER331): 5/group</td><td>ortho-Phenylphenol (OPP)
Induction: 0.4 g moistened with 0.2 ml water; challenge: 75% aqueous suspension.

Poitive: DER 331 espoxi resin: 10% induction and challenge.</td><td>Results:
<table><tr><th>Induction</th><th colspan="2">None</th><th>100% OPP</th><th>10% DER 331</th></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>24 h</td><td>0/5</td><td>1*/5</td><td>0/10</td><td>10**/10</td></tr><tr><td>48 h</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 (as stated in the study report)
**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>Gilbert, K.S. (1994b) (CA) B.6.2.6-02</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance, Dose levels, duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>sham control group.
Dose solutions were not analysed for homogeneity or dose confirmation.

Supportive only</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Skin sensitization in guinea pig, Method: Similar to OECD 406 (GPMT)
GLP: Not applicable.
Published study

Deficiences: Test substance not characterised. No individual results reported. Grade 1 reactions were omitted in the analysis of the test results
Supportive only</td><td>Guinea Pig
Outbred, females

Test group: 20 (4 compounds could be tested simultaneously)
Control group: 20 animals</td><td>ortho-Phenylphenol (OPP) and Sodium 2-phenylphenolate (SOPP)

Intradermal induction: 0.5 or 5%
Topical induction: 25%
Challenge: 5%
Rechallenge (1 positive animal treated with SOPP): 5%.</td><td>Results:
<table><tr><th rowspan="2">Test compound</th><th rowspan="2">Induction [%] (intradermal + topical)</th><th colspan="2">Frequency of positive challenge on day</th></tr><tr><th>21</th><th>28</th></tr><tr><td rowspan="2">OPP</td><td>0.5 + 25</td><td>0/20</td><td>–</td></tr><tr><td>5 + 25</td><td>0/20</td><td>–</td></tr><tr><td rowspan="2">SOPP</td><td>0.5 + 25</td><td>1*/20</td><td>1*/1</td></tr><tr><td>5 + 25</td><td>0/20</td><td>–</td></tr></table>* Only positive and doubtful positive animals were re-challenged

Only challenge days 21 and 28 responses were reported: it is not clear if the reported positive values correspond to the 48 and 72 h readings altogether, or only to the 72 h reading (which was the data used for statistics). Grade 1 reactions were omitted in the analysis of the test results.</td><td>Andersen, K.E. and Hamann, K. (1984) (CA) B.6.2.6-03</td></tr>
<tr><td>Skin sensitization in guinea pig, OECD 406 (1987) - Buehler method
GLP: Yes

Deviations: Only 10 animals in the treated group (required: ≥ 20 animals).
Only 5 animals in the control group (required: ≥ 10 animals). No justification was given for the use of a naive control group instead of a sham control group.
Dose solutions were not analysed for homogeneity or dose confirmation.

Supportive only</td><td>Guinea Pig
Hartley, males

Test group: 10
Positive control group: 10
Negative control groups (SOPP and DER 331): 5/group</td><td>Sodium ortho-phenylphenate (SOPP)

Induction: 0.5%
challenge: 0.1%

Poitive: DER 331 espoxi resin: 10% induction and challenge.</td><td>Results:
<table><tr><th>Induction</th><th colspan="2">None</th><th>0.5% SOPP</th><th>10% DER 331</th></tr><tr><th>Challenge</th><th>0.1% SOPP</th><th>10% DER 331</th><th>0.1% SOPP</th><th>10% DER 331</th></tr><tr><td>Time</td><td></td><td></td><td></td><td></td></tr><tr><td>24 h</td><td>0/5</td><td>1/5</td><td>0/10</td><td>9*/10</td></tr><tr><td>48 h</td><td>0/5</td><td>0/5</td><td>0/10</td><td>9**/10</td></tr></table>* Erythema grade 0.5 (very slight) in 5 animals, grade 1 (slight) in 2 animals and grade 2 (moderate) in 2 animals.
** Erythema grade 1 (slight) in 6 animals and grade 2 (moderate) in 3 animals.</td><td>Gilbert, K.S. (1994c) (CA) B.6.2.6-05</td></tr>
</table>

Table 37: Summary table of human data on skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Skin sensitization in humans, Published study 200 unselected human subjects (100/sex) **Supportive only**	*ortho*-Phenylphenol (**OPP**) and Sodium 2-phenylphenolate (**SOPP**) OPP: 5% in sesame oil SOPP: 5.0, 1.0, 0.5 and 0.1% (aqueous solutions)	1st application: 5 days in contact. 2nd application (3 weeks later): 48h in contact. Readings: after removal of both patches, and also days 3 and 8 after removal of the 2nd patch.	Results: OPP did not cause primary irritation when tested as a 5% solution in sesame oil nor did it cause any sensitisation. SOPP was found to be significantly irritating both at 5% and at 1% concentration. A 0.5% solution caused a very slight, simple irritation whereas 0.1% failed to produce any irritation. SOPP also failed to cause any sensitization when tested at a concentration of 0.1%.	Hodge, H.C. *et al*. (1952) (CA) B.6.2.6-04
Occupational medical surveillance on manufacturing plant personnel. *Report not provided*	*ortho*-Phenylphenol (**OPP**)	Occupational medical surveillance of workers potentially exposed to OPP is performed in 3-year intervals on a routine basis.	Results based on ca. 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) (CA) B.6.9.1
Allergic contact dermatitis due to *o*-phenylphenol. USA published report *Extracted from Previous DAR*	*ortho*-Phenylphenol (**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. Exposure to OPP was suspected to be through a germicidal agent with OPP in the first case (medical laboratory assistant), and a coolant with OPP (machinist).	Adamds, R.M. (1981) (CA) B.6.9.2
Contact Urticaria to *o*-phenylphenate USA published report *Extracted from Previous DAR*	*ortho*-Phenylphenol (**OPP**)	Single case. Unusual presentation of contact urticarial in the form of an inmediate 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 minits at the site where the preservative OPP had been placed. Similar challenge in 20 control subjects produced no reactions.	Tuer, W.F., James, W.D. and Summers, R.J. (1986) (CA) B.6.9.2
Contact sensitivity to *o*-phenylphenol in a coolant. Belgium published report *Extracted from Previous DAR*	*ortho*-Phenylphenol (**OPP**) sodium *ortho*-phenylphenate (SOPP)	Case report of one individual with dermatitis related to his work.	Machinist worked in contact with coolant liquid (containing OPP) and sometimes a cleanser (containing SOPP) was added to the coolant. Testing with coolant revealed sensitivity to OPP. OPP (1% in petrolatum) and the cleanser caused redness, oedema and vesicles.	Van Hecke, E. (1986) (CA) B.6.9.2
Epidemiological study Contact allergies caused by industrial biocides Germany (IVDK) published report *Extracted from Previous DAR*	*ortho*-Phenylphenol (**OPP**) was one of the test compounds	Patch test reactions to several industrial biocides (OPP was one of the tested ones). 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. Exposition for 48 h (in 732 patients) or 24 h (in 400	Results: 5 individuals (0.40%) showed positive reactions, 1 individual showed irritation, and 1 individual showed ambiguous result.	Geier *et al.* (1996) (CA) B.6.9.4

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		patients). Skin reaction was scored 72 h after application of the patch.		
Retrospective study based on data collected by the IVDK Germany (IVDK) published report *Extracted from Previous DAR*	*ortho*-Phenylphenol (**OPP**)	More than 40000 patch test reactions against a set of 24 medical preservatives in 2059 patients, recorded between 1989 and 1991, were analysed by computerised data processing. 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.	Results: 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) (CA) B.6.9.4
Dermatoses in metal workers (II). Allergic contact dermatitis Netherlands published report *Extracted from Previous DAR*	*ortho*-Phenylphenol (**OPP**)	Epidemiological study (in 10 metalworking factories): the prevalence of contact sensitisation was investigated in 286 metalworkers exposed to metalworking fluid (MWF). Patch tests were also performed with OPP (1% in petrolatum). 48 h exposure (occlusion). Scorings: 72 h after patches application. Several workers presented skin lesions at the time of the investigations.	Results: 8 workers of 286 showed contact allergy. None of these cases were related to OPP.	De Boer *et al.* (1989) (CA) B.6.9.4
Contact Allergy in Metal Workers – 1-year analysis based on data collected by IVDK Germany (IVDK) published report *Extracted from Previous DAR*	*Ortho*-phenylphenol (**OPP**)	Epidemiological study to investigate the prevalence of contact sensitisation in 424 metalworkers exposed to metalworking fluid (MWF). 2 test series: - Additives industrial fluids (included OPP) - common components of MWF 277 patients received an application of 1% OPP in petrolatum, Exposition for 48 h (occlusion). Scoring at 72 h after the patches were applied.	Results: 2 individuals showed a positive reaction (0.72%)	Uter *et al.* (1993) (CA) B.6.9.4
Patch testing with preservatives, antimicrobials and industrial biocides.	*ortho*-Phenylphenol (**OPP**)	The role of different preservatives (OPP included) in a large	Results: 33 subjects (0.3%) were positive. 59 subjects (0.5%) showed an irritative	Geier *et al.* (1998) (CA)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
(Results from a multicentre study) Germany (IVDK) published report *Extracted from Previous DAR*		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. Exposure for 24 or 48 h Readings 72 h after application	or questionable result.	B.6.9.4

Table 38: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

2.6.2.7.1 Short summary and overall relevance of the provided information on skin sensitisation

All the skin sensitization studies with ***ortho*-phenylphenol (OPP)** provided for the renewal of the active substance were already included and assessed in the previous DAR (2008).

Human data for skin sensitization was also included and assessed in the previous DAR (2008); however, in the dossier provided by the applicant for the renewal of the active substance, only the study of Hodge, H.C. *et al.* (1952; B.6.2.6-04) and data on occupational medical surveillance was included (updating the previous data), but no information has been included regarding direct observation and epidemiological studies, due to the fact that no new reports had been published since the first evaluation of OPP under Directive 91/414/EEC. However, RMS considers the published allergy reports involving contact allergy to OPP (submitted for the previous DAR) sufficiently relevant to be taken into account on the assessment of this hazard class.

The outcome of the three animal studies suggests no evidence of skin sensitization. Only one of these studies was considered acceptable in the previous DAR (Gilbert, K.S., 1994b; B.6.2.6-02); however, due to the fact that the study used only half of the required number of animals, together with the uncertainty of negative results in the Buehler assay of three induction applications (which are not considered to be conclusive to evaluate the skin sensitisation potential, as it may lead to a false negative outcome due to the low number of induction applications) the conclusion on the acceptability of this study changes in this revision, and is considered as supportive only.

Similar situation occurred with sodium ***ortho*-phenylphenate (SOPP)**, where a new sensitisation study has been provided (Gilbert, K.S., 1994c; B.6.2.6-05) which is considered acceptable by the applicant, but the abovementioned deviations have been detected also in this study (only half of the required number of animals were used, together with the uncertainty of negative results in the Buehler assay of three induction applications) and, therefore, the study is considered as supportive only.

Provided data for SOPP includes also two studies that used OPP and SOPP: a guinea pig study (B.6.2.6-03) and a human patch test (B.6.2.6-04), which are commented below, and were already included in the previous DAR. However, no medical or other data has been provided for SOPP regarding skin sensitization. Therefore, the following assessment includes both substances together.

The provided data on Guinea Pig Maximization Test with OPP and SOPP (Andersen, K.E. and Hamann, K., 1984; B.6.2.6-03) were used, is included in a publication that can be considered as supportive but not acceptable for classification purposes, due to relevant deficiencies: test substance not characterised, no individual results were reported; only challenge days 21 and 28 responses were reported, and it is not clear if the reported positive values correspond to the 48 and 72 h readings altogether, or only to the 72 h reading (which was the data used for statistics). Moreover, Grade 1 reactions were omitted in the analysis of the test results and therefore these data

were not regarded as sensitisation responses, nor were included in the publication.

Considering all this information, no conclusion on the classification can be drawn on the basis of the animal data.

As for the available human data, among negative results obtained in several studies, few positive skin sensitisation cases were also reported. This information should be considered carefully: 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 and SOPP are corrosive to the skin, and different exposure times and sometimes only one reading time point were used in the different tests).

The study of Hodge, H.C. *et al*. (1952; B.6.2.6-04) shows no evidence of skin sensitization after the application of OPP and SOPP to 200 unselected human subjects (both sexes). This publication can be considered as supportive information, since it's prior to any guidance, and it cannot be assumed that it was a properly conducted HRIPT (Human Repeat Insult Patch Test).

According to the available data on occupational medical surveillance (Leng, 2019, B.6.9.1) that used the data of c.a. 65 employees (examined every 3 years between 2004 and 2018), no indications of skin sensitisation for OPP among employees was observed.

Data collected on humans (B.6.9.2) include the description of 3 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 urticaria related with the OPP found in one of the components of a plaster.

Also epidemiological studies (B.6.9.4) were available for the previous DAR (2008). Altogether, these published epidemiological studies showed a low sensitizing potential of OPP, with positive reactions in 0.29% to 0.72% of the study subjects. The results obtained in these five studies should be assessed carefully. Most of the data obtained comes from metal workers 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 48 h depending on the study/test facility), what makes it more difficult to compare possible results, moreover if the same reading time (72 h after application) was considered to evaluate the scores, and there is not a second reading to help distinguish between irritant and sensitizing responses. Only in one study (Brasch *et al*, 1993) described different reading time points (after removal of the patch and on the following two days).

The validity of the available studies and the lack of a reliable maximization test, in the background of a certain (although low) sensitisation rate in humans was already discussed in the previous evaluation (PRAPeR Expert Meeting 59 (2008): "*Normally M&K test required. There was no adequate replacement test available, the Buehler test and M&K reported in the DAR were not valid or sufficient, but extensive human case reports indicate low percentages of sensitisation (0.3%). The lymph node assay performed with the formulation (although not accepted) was also negative. The EPA (2006) and ECB (26 ATP) did not propose sensitization classification, but the database for this decision was not known. The majority of experts agreed it should not be classified. As it was agreed it was not a sensitizer, a data gap for a further study was not identified*".

Therefore, a conclusion was reached on the no classification of OPP.

***Ortho*-Phenylphenol (OPP)** and **sodium *ortho*-phenylphenate (SOPP)** classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). No classification regarding skin sensitisation is included for any of these substances.

RMS deems this no-classification should be maintained, since no new human data or epidemiological tests on skin sensitisation with OPP or SOPP has been reported in the last 20 years. The few provided reports that showed (low) positive sensitization responses (in 0.29% to 0.72% of the study subjects) are dated previously to the ECB decision on no classification of the test substance (the last epidemiological study reporting positive data is dated in 1998). Different methods and guidelines to standardize skins sensitization tests (and the interpretation of the skin responses in such tests, to help distinguish between irritation and sensitization effects) have been developed since then, and no new epidemiological data reporting positive cases with OPP or SOPP is available.

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

None of the available animal data (negative results in all the provided studies) is considered as fully reliable.

Human data provided for the previous DAR (2008) shows a low sensitizing potential of OPP, with positive reactions in 0.29% to 0.72% of the study subjects. However, these reports were published before 2000 and no new positive data has been reported on human medical surveillance or epidemiological studies.

Guidance on the classification of the substance can be found in CLP Regulation (points 3.4.2.2.4.2 and 3.4.2.2.4.3):

3.4.2.2.4.2. Evidence from animal studies is usually much more reliable than evidence from human exposure. However, in cases where evidence is available from both sources, and there is conflict between the results, the quality and reliability of the evidence from both sources must be assessed in order to resolve the question of classification on a case-by-case basis. Normally, human data are not generated in controlled experiments with volunteers for the purpose of hazard classification but rather as part of risk assessment to confirm lack of effects seen in animal tests. Consequently, positive human data on skin sensitisation are usually derived from case-control or other, less defined studies. Evaluation of human data must therefore be carried out with caution as the frequency of cases reflect, in addition to the inherent properties of the substances, factors such as the exposure situation, bioavailability, individual predisposition and preventive measures taken. Negative human data should not normally be used to negate positive results from animal studies. For both animal and human data, consideration should be given to the impact of vehicle.

3.4.2.2.4.3. If none of the abovementioned conditions are met, the substance need not be classified as a skin sensitiser. However, a combination of two or more indicators of skin sensitisation as listed below may alter the decision. This shall be considered on a case-by-case basis.

(a) Isolated episodes of allergic contact dermatitis;

(b) epidemiological studies of limited power, e.g. where chance, bias or confounders have not been ruled out fully with reasonable confidence;

(c) data from animal tests, performed according to existing guidelines, which do not meet the criteria for a positive result described in section 3.4.2.2.3, but which are sufficiently close to the limit to be considered significant;

(d) positive data from non-standard methods;

(e) positive results from close structural analogues.

Once assessed the available data, RMS deems the quality and reliability of the evidence from human data is questionable and, therefore, there conditions to classify the substance are not met.

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

No classification for skin sensitization is required for ***ortho*-phenylphenol (OPP).**

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;</td><td>Induction: 0.4 g moistened with 0.2 mL water

Challenge: 75% aqueous suspension

Positive: DER 331</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 colspan="5">Time</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</td><td>B.6.2.6-02, 1994b</td></tr>
</table>

<table>
<tr><td>only 5 animals in the control group</td><td>epoxi resin: 10% induction and challenge</td><td>**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></td></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 Contact allergies caused by industrial biocides	OPP Exposition for 48h (in 732 patients) or 24h (in 400) Skin reaction was scored 72h after application of the patch.	Patch test reactions to several industrial biocides (OPP was one of the tested ones). 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	Geier *et al*., 1996 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)	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

	Scorings: 72h after patches application		
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
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).

2.6.2.8 Phototoxicity

Table 39: Summary table of studies on phototoxicity

Method, guideline, deviations[1] if any	Test substance, dose levels, duration of exposure	Results	Reference
***In vitro* phototoxicity study** OECD TG 432 (2004) EC Method B.41 GLP: Yes **Study acceptable**	Species : Mouse System : Fibroblast cell line BALB/c 3T3 (clone A31) *In vitro* Purity. 99.9% Solvent: DMSO and EBSS Concentrations: 7.81, 15.63, 31.25, 62.50, 125.00, 250.00, 500.00 and 1000.00 µg/mL Negative control: 1% DMSO in	Results: Pronounced cytotoxicity starting from 125 µg/mL both ± UVA The corresponding calculated EC_{50} values are 93.47 µg/mL (-UVA) and 84.37 µg/mL (+UVA) PIF = 1.12 MPE < 0.001	**Leuschner, J., 2018 (AR) B.6.2.7**

Method, guideline, deviations[1] if any	Test substance, dose levels, duration of exposure	Results	Reference
	EBSS Positive control: Chlorpromazine (concentrations: m0.01, 0.10, 1.0 and 10.0 µg/mL) Incubation: 60 min in the dark (5% CO2, 37 ± 1°C and a relative humidity of 95% ± 5%) followed by: (+UVA): 8.90 min at 9.36 mW/cm^2 (-UVA): 8.90 min in the dark	**Not phototoxic**	

Table 40: Summary table of human data on phototoxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 41: Summary table of other studies relevant for phototoxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

No phototoxicity potential was observed in the available study, performed with ***ortho*-phenylphenol (OPP)**.

There is no phototoxicity test with **sodium *ortho*-phenylphenate (SOPP)**. However, the phototoxicity test with OPP (Leuschner, 2018; B.6.2.7) is considered representative for SOPP as well and, therefore, previous conclusion applies for SOPP

2.6.2.9 Aspiration hazard [equivalent to section 10.13 of the CLH report template]

Table 42: Summary table of evidence for aspiration hazard

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

2.6.2.9.1 Short summary and overall relevance of the provided information on aspiration hazard

No evidence of aspiration hazard of *ortho*-phenylphenol (OPP) or sodium *ortho*-phenylphenate (SOPP) was found in the provided data

2.6.2.9.2 Comparison with the CLP criteria regarding aspiration hazard

Although the definition of aspiration in section 3.10.1.2 of Regulation (EC) No. 1272/2008 includes the entry of solids into the respiratory system, classification criteria for this hazard is established for liquid, aerosol and mist forms of a substance or a mixture.

ortho-Phenylphenol (OPP) is presented in a solid (flakes) form and, therefore, no aspiration toxicity hazard is expected.

2.6.2.9.3 Conclusion on classification and labelling for aspiration hazard

Data available indicates that ***ortho*-phenylphenol (OPP)** does not require classification for aspiration hazard.

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.

2.6.2.10 Specific target organ toxicity-single exposure (STOT SE) [equivalent to section 10.11 of the CLH report template]

Table 43: Summary table of animal studies on STOT SE (specific target organ toxicity-single exposure)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Acute oral toxicity study in rats No guidelined, but similar to OECD TG 401 (1987) GLP: No Species: Rat Strain: Wistar 10 males/dose Deviations: Test material not characterised; animals not fasted; dosing into duodenum; necropsy by random sampling; individual bw not reported. **Supportive only** *Guideline value for classification: STOT SE 1 ≤ 300 mg/kg bw/day STOT SE 2≤ 2000 mg/kg bw/day and >300 mg/kg bw*	**OPP (Preventol O extra)** Purity: Not specified Vehicle: Lutrol (polyethylene glycol) Oral (dosing into duodenum) Single dose Doses: 1500, 2000, 3100, 4000, 4500 and 5000 mg/kg bw 14-day observation period	*Only effects relevant for STOT SE are presented (see also section 2.6.2.1 for more study details)* **Clinical signs**: Several clinical signs were observed (anaesthesia, impaired general condition, abdominal recumbency and lateral recumbency) in all dose groups.	Löser, E. (1981) **B.6.2.1-01 (AS)**
Acute oral toxicity study in rats No guidelined, but comparable to OECD TG 401 (1987) GLP: No Species: Rat Strain: Not indicated 10-20 males/dose Deviations: only a brief summary,	**OPP** Purity: >98% Vehicle: olive oil/gum acacia Oral (gavage) Single dose Doses: 1600, 2000, 2400, 2800, 3000, 3200 and 4000 mg/kg bw Observation until recovery (usually about 2 weeks)	*Only effects relevant for STOT SE are presented (see also section 2.6.2.1. for more study details)* **Clinical signs**: Not reported, but death from 2000 mg/kg bw appeared to be due to progressive depression terminating in respiratory failure.	Hodge, H.C. *et al.* (1952) **B.6.2.1-03 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
batch not reported; strain not specified; incomplete test method description; individual bw only recorded at the beginning; necropsy not performed. **Supportive only** *Guideline value for classification: STOT SE 1≤ 300 mg/kg bw/day STOT SE 2≤ 2000 mg/kg bw/day and >300 mg/kg bw*			
Acute oral toxicity study in mice Not possible to check test method GLP: No Species: Mouse Strain: ddY 10 mice/sex/dose Deviations: publication written in Japanese, only abstract and results table/graphs in English; not possible to check the method; purity not reported. **Supportive only** *Guideline value for classification: STOT SE 1≤ 300 mg/kg bw/day STOT SE 2≤ 2000 mg/kg bw/day and >300 mg/kg bw*	**OPP** Purity: not indicated Vehicle: propylene glycol Oral gavage Single dose Doses: 0, 414, 538, 700, 910, 1183, 1538 and 2000 mg/kg bw. 14-day observation period	*Only effects relevant for STOT SE are presented (see also section 2.6.2.1 for more study details)* **Clinical signs:** reported information was not detailed (*e.g.* onsent of symphtoms or data by groups). Decrease of spontaneous movement, limb position, staggering gait and low respiratory rate were the main clinical symptoms.	Taniguchi, Y. *et al.* (1981) **B.6.2.1-04 (AS)**
Acute oral toxicity study in rats OECD TG 401 (1987) GLP: Yes Species: Rat Strain: Fisher 344 5 rats/sex/dose **Acceptable**	**OPP** Purity: 99.9% Vehicle: Corn oil Oral (gavage) Single dose Doses: 500, 2500 and 5000 mg/kg bw 14-day observation period	*Only effects relevant for STOT SE are presented (see also section 2.6.2.1 for more study details)* **Clinical signs**: lacrimation, salivation, chromorhinorrhea, laboured respiration, decreased activity, lateral recumbency and urine and faecal soiling in the perineal area in both males and females from 2500 mg/kg bw. **Necropsy:** 5000 mg/kg bw: - Death on day 1 (5 females, 2 males): no gross lesions. - Death on day 2 (2 males): hemolysed blood in	Gilbert, K.S. and Crissman, J.W. (1994) **B.6.2.1-05 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Guideline value for classification: *STOT SE 1 ≤ 300 mg/kg bw/day* *STOT SE 2≤ 2000 mg/kg bw/day and >300 mg/kg bw*		the digestive tract. - Death on day 3 (1 male): perineal soiling and lung congestion lesions. 2500 mg/kg bw: - Death on day 2 (1 male, 1 female): hemolysed blood in the digestive tract. - Death on day 3 (1 male, 1 female): perineal soiling. - Surviving males: fibrous adhesions between the serosa of the non-glandular portion of the stomach and liver. - Surviving females: no gross lesions. 500 mg/kg bw: - No gross lesions.	
Acute oral toxicity study in mice Not possible to check test method. GLP: No Species: Mouse Strain: IRC 10 mice/sex/dose Deviations: publication written in Japanese, only brief abstract and results table/graphs in English; it is not possible to check the method. **Supportive only** *Guideline value for classification:* *STOT SE 1 ≤ 300 mg/kg bw/day* *STOT SE 2≤ 2000 mg/kg bw/day and >300 mg/kg bw*	**OPP** Purity: 98% Vehicle: Olive oil Oral Single dose Doses: 1000, 1500, 2250, 3375, 5063 and 7594 mg/kg bw 14-day observation period	*Only effects relevant for STOT SE are presented (see also section 2.6.2.1 for more study details)* **Clinical signs**: reported information was not detailed (*e.g.* onsent of symphtoms or data by groups). Decrease of motor activity, sedation and lacrimation were the main clinical symptoms.	Tayama, K. *et al.* (1983) **B.6.2.1-06 (AS)**
Acute oral toxicity study in rats OECD TG 401 (1987) GLP: Yes Species: Rat Strain: Fisher 344 5 rats/sex/dose **Acceptable** *Guideline value for classification:* *STOT SE 1 ≤ 300 mg/kg bw/day* *STOT SE 2≤ 2000*	**SOPP** Purity: 99.1% Vehicle: unclear Oral (gavage) Single dose Doses: 100, 500, 1000 and 5000 mg/kg bw 14-day observation period	*Only effects relevant for STOT SE are presented (see also section 2.6.2.1 for more study details)* **Clinical signs**: lacrimation, salivation, chromorhinorrhea, laboured respiration, decreased activity, lateral recumbency, incoordination, decreased muscle tone, mouth breathing and urine and fecal soiling in the perineal area began 30 min after treatment Most clinical signs resolved during the observation period (a few signs persisted in 1 male survivor at 1000 mg/kg bw and 1 female survivor at 500 mg/kg bw). **Necropsy:** Rats that died during the observation period had one or more of the following findings: hemolyzed blood in the digestive tract, perineal soiling, general visceral congestion, decreased amount of fat, pale liver, congested lungs, bloody urine and congestion, erosions and/ or ulcers,	Gilbert, K.S. and Stebbins, K.E. (1994) **B.6.2.1-07 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
mg/kg bw/day and >300 mg/kg bw		hemorrhage, or hyperemia of the stomach. Gross observations of the stomach and digestive tract were consistent with stress-induced alterations. There were no treatment-related gross pathologic observations in any of the surviving rats.	
Acute oral toxicity study in rats No guidelined, but similar to OECD TG 401 (1987) GLP: No Species: Rat Strain: Wistar 5 animals/sex/ dose Deviations: Test material not characterised; animals not fasted; necropsy was not performed; individual bw not reported. **Supportive only** *Guideline value for classification: STOT SE 1 ≤ 300 mg/kg bw/day STOT SE 2≤ 2000 mg/kg bw/day and >300 mg/kg bw*	**SOPP (Preventol ON extra)** Purity: Not specified Vehicle: water Oral (gavage) Single dose Doses: 1000, 1300, 1500, 2000, 2200 and 2500 mg/kg bw 14-day observation period	*Only effects relevant for STOT SE are presented (see also section 2.6.2.1 for more study details)* **Clinical signs**: 1300 mg/kg bw: narcosis and decline in general condition in all rats on day 1 and 2, persisting in surviving rats up to day 5. 1500 mg/kg bw: narcosis and a decline in general condition in all rats on day 1 and 2, persisting in 4/5 surviving males and 2/5 females up to day 5. 2000 mg/kg bw: narcosis and a decline in general condition in all rats on day 1 and 2, persisting in 3/5 surviving males up to day 5 and in 3/5 surviving females up to the end of the observation period. 2200 mg/kg bw: narcosis and a decline in general condition in all rats on day 1 and 2, persisting in the surviving males up to the end of the observation period. 2500 mg/kg bw: narcosis and a decline in general condition in all rats on day 1 and 2, persisting in 1 female until death on day 3.	Löser, E. (1980) **B.6.2.1-08 (AS)**
Comet assay *in vivo* Pre-guidaline GLP: Yes Species: Mouse Strain: CD-1 (Crl: CD-1(ICR)BR, SPF) 4 males/group Deviations from OECD TG 489 (2016): Only 4 animals used; duration of treatment less than 2 days; no justification for a viscous vehicle; total cells per organ less than 150, no HCD reported.	**OPP (Preventol O-extra)** Purity: 99.8 % Vehicle: olive oil Oral (gavage) Single dose Doses: 0, 250 and 2000 mg/kg Volume: 10 mL Sacrifice 3, 8 and 24 h after treatment. Sampling in liver and kidney	*Only effects relevant for STOT SE are presented (see also section 2.6.4 for more study details)* **Clinical signs:** apathy, semi-anaesthesised state, roughened fur, pallor, staggering gait, sternal recumbency, spasm, shivering, languor, wide-legged gait and slitted eyes at 2000 mg/kg bw. 2 mice at 2000 mg/kg bw died during the test period.	Brendler-Schwaab, S. (2000) **B.6.4.2.2-01 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Supporting information *Guideline value for classification:* *STOT SE 1 ≤ 300 mg/kg bw* *STOT SE 2 ≤ 2000 mg/kg bw and >300 mg/kg bw*			
Developmental toxicity study in rat No guidelined GLP: No. Species: Rat Strain: Wistar 11 to 20 females/dose **Supportive only** *Guideline value for classification:* *STOT SE 1 ≤ 300 mg/kg bw* *STOT SE 2 ≤ 2000 mg/kg bw and >300 mg/kg bw*	**OPP** Purity: 99.7% Oral (gavage) 0, 150, 300, 600 and 1200 mg/kg bw/day Exposure from GD 6 to GD15 (inclusive)	*Only effects relevant for STOT SE are presented (see also section 2.6.6.2 for more study details)* **Clinical signs in dams:** Ataxia for several hours from 300 mg/kg bw, the severity of which was dose-dependent.	Kaneda *et al.* (1978) **B.6.6.2-01 (AS)**

Table 44: Summary table of human data on STOT SE (specific target organ toxicity-single exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 45: Summary table of other studies relevant for STOT SE (specific target organ toxicity-single exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

2.6.2.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure (STOT SE)

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture. Relevant information for STOT SE is covered by acute toxicity studies in form of clinical observations, and macroscopic and microscopic pathological examination that can reveal hazards that may not be life-threatening but could indicate functional impairment. Effects of other single dose studies or repeated dose studies (first dosing effects) are also considered for STOT SE.

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 recumbency and lateral recumbency 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 recumbency and urine and faecal soiling in the perineal area were observed in both sexes from 2500 mg/kg bw, and necropsy findings occurred also from this dose (hemolysed blood in the digestive tract. perineal soiling, fibrous adhesions between the serosa of the non-glandular portion of the stomach and liver).
Besides these acute toxicity studies, information on STOT SE was also obtained from a developmental toxicity test in rats (B.6.6.2-01), in which ataxia for several hours was observed in dams from 300 mg/kg bw. The severity of this ataxia was dose-dependent. This last study was also considered as supportive only.

Studies in mice

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-anaesthesised state, roughened fur, pallor, staggering gait, sternal recumbency, spasm, shivering, languor, wide-legged gait and slitted eyes were observed at 2000 mg/kg bw (B.6.4.2.2-01).
However, these three studies were all considered only as supportive.

2.6.2.10.2 Comparison with the CLP criteria regarding STOT SE (specific target organ toxicity-single exposure)

STOT SE 1 and 2

STOT SE Categories 1 and 2 are assigned on the basis of findings of ‘significant’ or ‘severe’ toxicity. In this context ‘significant’ means changes, which clearly indicate functional disturbance or morphological changes, which are toxicologically relevant. ‘Severe’ effects are generally more profound or serious than ‘significant’ effects and are of a considerably adverse nature with significant impact on health. Both factors have to be evaluated by weight of evidence and expert judgement.

For OPP, the oral route has been considered the more relevant route for STOT SE. None of the effects observed were considered as enough 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 amount above the range for classification on these categories: Guidance range of value for classification as STOT SE Category 2 is ≤ 2000 mg/kg bw and >300 mg/kg bw, but lacrimation, salivation, chromorhinorrhea, laboured respiration, decreased activity, lateral recumbency and urine and faecal soiling in the perineal area were observed only from 2500 mg/kg bw. This dose is close to the rat LD_{50} set in 2733 mg/kg bw.

STOT SE 3

STOT SE 3 includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2.

According to the available results, some narcotic effects were observed after administration of OPP:

- In a supportive acute oral study in rats (B.6.2.1-01) anaesthesia, impaired general condition, abdominal recumbency and lateral recumbency were reported from 1500 mg/kg bw. An LD_{50} value of 2980 mg/kg bw (males) was set.

- In a supportive acute oral study in rats (B.6.2.1-03): progressive depression terminating in respiratory failure was observed from 2000 mg/kg bw.

- In the acceptable acute oral study in rats (B.6.2.1-05): decreased activity and lateral recumbency were observed from 2500 mg/kg bw.

- In a supportive developmental toxicity test in rats (B.6.6.2-01): ataxia for several hours was observed in dams from 300 mg/kg bw.

- In a supportive acute oral study in mice (B.6.2.1-04): decrease of spontaneous movement, staggering gait and low respiratory rate were observed.

- In a supportive acute oral study in mice (B.6.2.1-06): motor activity and sedation were reported.

- In a supportive Comet assay in mice (B.6.4.2.2-01), apathy, semi-anaesthesised state, staggering gait, sternal

recumbency, languor, wide-legged gait and slitted eyes were observed at 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 LD_{50}. These effects should be considered as covered by the adopted oral acute toxicity classification.

No data regarding classification as STOT SE category 3 (respiratory tract irritation) is available.

The previous DAR justified the maintenance of classification of OPP as STOT SE 3 (H335) based on the assumption that because of its proven severe irritation effects, it can be reasonably assumed that *ortho*-phenylphenol (OPP) is irritating to the airways when inhaled in high concentrations. However, this argument does not comply with the criteria established in the actual Regulation (EC) No. 1272/2008.

***Ortho*-Phenylphenol (OPP)** classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). Classification regarding STOT SE is included as: specific target organ toxicity – single exposure, category 3 (STOT SE; H335).

2.6.2.10.3 Conclusion on classification and labelling for s (specific target organ toxicity-single exposure)

As listed in Annex VI of Regulation (EC) No. 1272/2008, ***ortho*-phenylphenol (OPP)** is classified as: specific target organ toxicity – single exposure, category 3 (STOT SE; H335). RMS deems this classification should be deleted. Therefore, no classification is proposed for this hazard class (data conclusive but not sufficient for classification).

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.

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

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

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.**

2.6.3 Summary of repeated dose toxicity (short-term and long-term toxicity) [section 10.12 of the CLH report]

2.6.3.1 Specific target organ toxicity-repeated exposure (STOT RE) [equivalent to section 10.12 of the CLH report template]

Table 46: Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure)

Method **Guideline.** **Deviations if any/Acceptability** **Species, strain** **Sex** **No/group**	**Test substance.** **Route of exposure** **Dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** **[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)]**	**Reference**

<table>
<tr><th>Method
Guideline.
Deviations if any/Acceptability
Species, strain
Sex
No/group</th><th>Test substance.
Route of exposure
Dose levels, duration of exposure</th><th>Results
- NOAEL/LOAEL
- target tissue/organ
- critical effects at the LOAEL

[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)]</th><th>Reference</th></tr>
<tr><td colspan="4">Subacute oral toxicity</td></tr>
<tr><td>1-month dietary.
No guideline.
Supportive only.
Rats of unspecified strain.
Females.
5/ Dose group.</td><td>OPP (98% purity)
Dietary,
0, 2, 3, 4, 5, 10% of diet’s weight, for 1-month (approximately equivalent to 0, 2000, 3000, 4000, 5000 and 10000 mg/kg bw/day).</td><td>Mortality: 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>10,000</td><td>5/5</td></tr></table>Clinical signs: 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, H.C. et al. (1952)
(CA)
B.6.3.1-01</td></tr>
<tr><td>32-day oral.
No guideline.
Supportive only.
White rat.
Males.
15/Dose group.</td><td>OPP,
Oral gavage
0, 2, 20, 200 mg/kg bw/day, for 32-days.</td><td>There were no reported adverse attributable to OPP administration.

-LOAEL > 200 mg/kg bw/day.
-NOAEL = 200 mg/kg bw/day.
-Target organs/tissues were not identified.
-Critical effect at the LOAEL: n.a.</td><td>Macintosh, F.C., (1945)
(CA)
B.6.3.1-02</td></tr>
<tr><td>13-day oral.
EPA FIFRA 83-3(b) but checked for compliance with OECD 407.
Deviations: only females and only 2 animals per dose were used.
Haematology, clinical chemistry, and histopathology not conducted.
Supportive only.
New Zealand White rabbit.
Females.
2/Dose group.</td><td>OPP (99.77% purity)
Oral gavage,
0, 100, 500 or 1000 mg/kg bw/day, for 13 days.</td><td>Mortality:
▪ In the high dose group, 1 rabbit died on test day 8 and 1 rabbit was sacrificed moribund on test day 10.
Clinical signs :
▪ Decreased amount of faeces was observed in all the treated 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.

1<u>000 mg/kg bw/day:</u>
▪ ↓ final bw (25%)
▪ Decrease in food consumption (2/2, ns; no numerical data available)

5<u>00 mg/kg bw/day:</u>
▪ ↓ final bw (6.3%, ns)
▪ ↑ abs./rel, kidney wt. (11.5%, ns/19.2%, ns).
▪ ↓ abs./rel, liver wt. (20%, ns, ndr/15%, ns, ndr).
▪ Decrease in food consumption (2/2, ns; no numerical data available).

1<u>00 mg/kg bw/day:</u>
▪ ↓ abs./rel, liver wt. (26%, ns, ndr/24%, ns ndr).

-LOAEL = 500 mg/kg bw/day.
-NOAEL = 100 mg/kg bw/day.</td><td>Zablotny, C.L., Breslin, W.J., Kociba, R.J.
(1991a)
(CA)
B.6.3.1-03</td></tr>
</table>

Method **Guideline.** **Deviations if any/Acceptability** **Species, strain** **Sex** **No/group**	**Test substance.** **Route of exposure** **Dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** **[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)]**	**Reference**
		-Target organs/tissues were not identified. **-Critical effect at the LOAEL**: ↓ in bw, bw gain and amount of fat and ↑abs. and rel. kidneys weights.	
4-week oral. No guideline. S**upportive only.** Beagle dogs. Both sexes. 2/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. Range finding: Palatability study with doses from 300 to 900 mg/kg bw/day.	*General observations:* ▪ Dose-related emesis in all dogs (♂ and ♀) treated with ≥200 mg/kg bw/day ▪ No deaths occurred throughout the study at any dose tested. *Bodyweight* ▪ No differences in bw were found compared with controls. *Haematology:* **<u>300 mg/kg bw/day:</u>** ▪ ↓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.) **<u>200 mg/kg bw/day:</u>** ▪ ↓RBC (11%, n.s.) in ♂. ▪ ↓HCT (9%, n.s.) in ♂. **<u>100 mg/kg bw/day:</u>** ▪ ↓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 (♂,♀).	Cosse *et al.* (1990) (CA) B.6.3.1-04
Subchronic oral toxicity			

Method **Guideline. Deviations if any/Acceptability** **Species, strain** **Sex** **No/group**	**Test substance.** **Route of exposure** **Dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** **[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)]**	**Reference**
3-month dietary. No guideline. **Supportive only.** Rats of unspecified strain. Both sexes. 12/ Dose group.	OPP (≥98% purity) Dietary 0, 0.1, 0.3, 1.0, 2.0%, of diet's weight [equivalent to: 0, 100, 300, 1000 and 2000 mg/kg bw/day (calculated for young rats)], for 1-month.	*Mortality***:** no significant difference between the mortality in the dosage groups and in the control group**.** **2000mg/kg bw/day (2% w/w):** ▪ Slight growth retardation (no detailed data provided in the study). ▪ ↑ liver, kidney and spleen wt. (ns). in some rats (no numerical data available). **1000mg/kg bw/day (1% w/w):** ▪ ↑ liver, kidney and spleen wt. in some rats (no numerical data available). -**LOAEL** = 2% (≈ 2000 mg/kg bw/day) -**NOAEL** = 1% (≈ 1000 mg/kg bw/day) -Target organs/tissues were not identified. -**Critical effect at the LOAEL:** ↓ in bw (♂,♀)	Hodge *et al.* (1952) (CA) B.6.3.2-01
6-month dietary. No guideline. **Supportive only.** Rats of unspecified strain. Both sexes. 12/ Dose group.	OPP (≥98% purity) Oral gavage 0, 50, 100, 200, 500 mg/kg bw/day, 5 days per week for 6 months.	*Mortality***:** it was low and unrelated to dosage. (No more information was available in the study) **500 mg/kg bw/day:** ▪ ↑ liver and kidney wt. (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 wt. (♂,♀).	
13-week dietary. No guideline but it is similar to OECD 408. Deviations: no neurobehavioral examinations, no detailed reporting. Supportive only **(Reliable).** F344/DuCrj rats. Both sexes. 10/sex/dose.	OPP (98% purity) Dietary 0, 0.156, 0.313, 0.625, 1.25, 2.5%, in diet, (equivalent in ♂/♀ to: 0, 182/ 202, 391/411, 761/ 803, 1669/1650, and 2798/3014 mg/kg bw/day respectively); for 13-weeks.	*Mortality:* ▪ In the high dose group, 2 ♂ died on day 4 and 1♀ died on day 8. **2.5%, ♂/♀ (2798/3014 mg/kg bw/day):** Bodyweight and food/water consumption: ▪ ↓ bw in ♂/♀ [throughout the study (from 27 to 44%/from 20 to 30%)]*. ↓ in terminal bw in ♂/♀ (11%,/22%). ▪ ↓ bw gain in ♂/♀ (first week 35/31% for ♂/♀)] ▪ ↓ food consumption (abs. wt.) in ♂/♀ [week 0 (83%/80%), week 3 (22%/ 23%) , week 6 (27%/18%), week 9 (29%/-) and week 12 (27%/-)]** ▪ ↓ water consumption (abs. wt.) 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, ns) and week 13 (1/8 Vs. 0/8 in controls, ns)] ▪ ↓ pH in ♂/♀ [week 9 and week 13]. Haematology: ▪ ↓ RBC in ♂ (5%). ▪ ↓ Hg in ♂/♀ (6.8%/6%). ▪ ↓ MCV in ♀ (2%). ▪ ↓ MCH in ♂/♀ (2%, ndr/7%).	Iguchi *et al.* (1984) (CA) B.6.3.2-02

Method **Guideline. Deviations if any/Acceptability** **Species, strain** **Sex** **No/group**	**Test substance.** **Route of exposure** **Dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** **[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)]**	**Reference**
		▪ ↓ MCHC in ♂ (5%). Organ wt.: ▪ Liver: ↑ abs. wt. in ♀ (17%, ndr) and ↑ rel. wt. in ♂/♀ (20%/33%). ▪ Thymus: ↓ abs. wt. in ♂/♀ (24%, ndr/9%, ndr) ▪ Spleen: ↓ abs. wt. in ♂/♀ (14%/9%, ndr) and ↑ rel. wt. in ♂ (9%). ▪ Kidney: ↑ rel. wt. in ♂/♀ (25%/15%). ▪ Adrenals: ↓ abs. wt. in ♂ (15%, ndr) and ↑ rel. wt. in ♂/♀ (13%, ndr/10%, ndr). ▪ Bladder: ↑ rel. wt. in ♂ (60%). Histopathology ▪ Inflammation of the kidneys in ♂/♀. ▪ Abnormal growth in the bladder mucosa in ♂. **1.25%, ♂/♀ (1669/1650 mg/kg bw/day):** Bodyweight and food/water consumption: ▪ ↓ bw 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, ns)] Haematology: ▪ ↓ Hg in ♀ (4%). ▪ ↓ MCH in ♀ (3%). Organ wt.: ▪ Liver: ↑ rel. wt. in ♂/♀ (11%/13%). ▪ Kidney: ↑ rel. wt. in ♂ (6%). ▪ Bladder: ↑ abs. wt. in ♂ (40%, ndr) and ↑ rel. wt. in ♂ (49%). Histopathology ▪ Abnormal growth in the bladder mucosa in ♂. **0.65%, ♂/♀ (761/ 803 mg/kg bw/day):** ▪ Liver: ↑ rel. wt. in ♂ (7%). ▪ Thymus: ↓ rel. wt. in ♂ (13%, ndr) and ↑ rel. wt. in ♀ (10%, ndr). ▪ Kidney: ↑ rel. wt. in ♂ (4%). **0.313%, ♂/♀ (**391/411 **mg/kg bw/day):** ▪ Liver: ↑ abs./rel. wt. 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. **These percentages have been roughly extrapolated from graphical data and are only an estimation.* ***only food/water consumption from one of every third week is reported here*	
One-year oral. No guideline but it is similar to OECD 409. Deviations: Only 4 animals per	OPP (99.77% purity) Oral gavage 0, 30, 100, 300 mg/kg bw/day, for 52-weeks.	*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.	Cosse *et al.* (1990) (CA) B.6.3.2-03

Method **Guideline.** **Deviations if any/Acceptability** **Species, strain** **Sex** **No/group**	**Test substance.** **Route of exposure** **Dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** **[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)]**	**Reference**
dose level, source of bone sample not specified, sternum and femur are required by the guideline, not suitable administration of test substance. **Supportive only.** Beagle Dogs. Both sexes. 4/sex/dose.	Range finding: See B.6.3.1-04	**<u>300 mg/kg bw/day:</u>** Bodyweight: ▪ ↓ Terminal bw 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 (♂/♀).	
One-year, oral No guideline but it is similar to OECD 409. Deviations: Only 1 or 2 animals per dose level were used, test substance not characterised, no individual data or group averages. No clinical chemistry. **Supportive only.** Dogs of unspecified strain/ (mongrels). Both sexes. 1 to 2 animals per dose level	OPP (≥98% purity) 0, 20, 200, 500 mg/kg bw/day, for 1-year.	*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. **<u>500 mg/kg bw/day</u>** *Organ weight:* ▪ ↑ kidney wt. ♂ (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) (CA) B.6.3.2-04
Other routes			
21-day, dermal. It follows the following guidelines:	OPP (99.82% purity) Dermal 0, 100, 500, 1000 mg/kg	**<u>1000 mg/kg bw/day:</u>** *Gross pathology:* ▪ ↑ Incidence of local skin irritation in ♂ (2/5 vs. 0/5 in control) and ♀ (5/5 vs. 0/5 in control).	Zempel & Szabo (1993) (CA)

Method **Guideline. Deviations if any/Acceptability** **Species, strain** **Sex** **No/group**	**Test substance.** **Route of exposure** **Dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** **[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)]**	**Reference**
EPA FIFRA 82-2, MAFF Subchronic Dermal Toxicity Study, and OECD Guideline 410. Deviations: adrenal weights not determined, some clinical chemistry parameters suggested by OECD 410 were not evaluated. **Accepted.** Fischer 344 rats. Both sexes.. 5/sex/dose.	bw/day, 5 days/week for 21-days. The test material was ground to a fine powder and applied without vehicle under occlusive dressing. Range finding: A probe study using 2 male and 2 female Fischer 344 rats was performed to verify that the test material administered at **1000** mg/kg did not produce any severe adverse systemic or dermal effects.	*Histopathology:* ▪ ↑ Incidence of hyperkeratosis and acanthosis in ♂ (3/5 vs. 0/5 in control) and ♀ (4/5 vs. 0/5 in control). **<u>500 mg/kg bw/day:</u>** *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 e systemic LOAEL**: No systemic effects in any group. -Target organs/tissues were not identified.	B.6.3.3-01
4-week, dermal. No guideline but it is similar to OECD 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. **Supportive**	OPP (> 99% purity) Dermal 0, 5.95, 11.4, 20.8, 35.7, 55.5 mg/0.1 mL acetone, 3 days/week for 4-weeks (Equivalence between amount of test substance applied and dose level in mg/kg bw/day was not reported). *This is a range finding study for the carcinogenicity dermal study B.6.5-05.*	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 (1986) (CA) B.6.3.3-02

Method Guideline. Deviations if any/Acceptability Species, strain Sex No/group	Test substance. Route of exposure Dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [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)]	Reference
only. Swiss Webster CF W mice. Both sexes. 10/sex/dose.			

Table 47: Summary table of human data on repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 48: Summary table of other studies relevant for repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of study/data Species/strain No of animals	Test substance Route of exposure Dose levees, duration of exposure.	Observations	Reference
Long-term toxicity and carcinogenicity*			

Type of study/data Species/strain No of animals	**Test substance Route of exposure Dose levees, duration of exposure.**	**Observations**	**Reference**
Combined Chronic Toxicity/carcinogenicity. Fischer 344rats. Both sexes. 20/sex and dose in the 1 year-group. 50/sex and dose in the 2 year-group. **Acceptable** *See table 53 for more information.*	OPP, (purity 99.7-100%) 0, 800, 4000, 8000/10,000 ppm for ♂/♀ (39/49, 200/248 and 402/647 mg/kg bw/day for ♂/♀) for 2-years.	*Only effects relevant for STOT RE* **8000/10000 ppm** ♂/♀ (402/647 mg/kg bw/day) 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) Neoplastic changes: *Urinary bladder* ▪ ↑ Incidence of transitional cell carcinomas in ♂ at 24 months (34/50 vs. 0/50 in controls) ▪ ↑ Incidence of papillomas in ♂ at 12 months (6/20 vs. 0/20 in controls) and at 24 months (6/50 vs. 0/50 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). *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) **4000 ppm** (200/248 mg/kg bw/day ♂/♀) Gross pathology: ▪ ↑ Incidence of urinary bladder masses in ♂ (4% vs. 0% in controls; n.s.). Neoplastic changes: *Urinary bladder* ▪ ↑ Incidence of transitional cell carcinomas in ♂ at 24 months (2/50 vs. 0/50 in controls; n.s.). Non-neoplastic changes:	Wahle & Christenson (1996) (CA) B.6.5-02

Type of study/data Species/strain No of animals	Test substance Route of exposure Dose levees, duration of exposure.	Observations	Reference
		Urinary bladder ▪ ↑ Incidence of simple hyperplasia in ♂ at 24 months (6/50 vs. 2/50 in control; n.s.). -Systemic LOAEL= 4000 ppm (200 mg/kg bw/day). -Systemic NOAEL= 800 ppm (39 mg/kg bw/day). -Critical effect at the LOAEL: structural alterations in the urinary bladder (♂). -Neoplastic LOAEL= 8000 ppm (402 mg/kg bw/day). -Neoplastic NOAEL= 4000 ppm (200 mg/kg bw/day). -Critical effect at the LOAEL: neoplasms (malignant and benign) in the urinary bladder (♂). **-Target tissue/organ: Urinary bladder.**	
Dietary in, mouse. B6C3F1 mice. 60/sex and dose. **Acceptable** *See table 53 for more information.*	OPP (purity 99.88%) Dietary 0, 250, 500, 1000 mg/kg bw/day, for 2-years	*Only effects relevant for STOT RE* **1000 mg/kg bw/day** Neoplastic changes *Liver* ▪ ↑ Hepatocellular adenoma in ♂ (82%; 41/50 animals vs 54%; 27/50 in controls). ▪ ↑ Hepatocellular carcinoma in ♂ (24%; 12/50 animals vs 22%; 11/50 in controls; n.s.; ndr). ▪ ↑ Malignant hepatoblastoma in ♂ (6%; 3/50 animals vs 0%; 0/50 in controls; n.s.; ndr). ▪ ↑ Hepatocellular carcinoma/ hepatoblastoma in ♂ (30%; 15/50 animals vs 22%; 11/50 in controls; n.s; ndr). ▪ ↑ Hepatocellular adenoma/ carcinoma/ hepatoblastoma in ♂ (86%; 43/50 animals vs 64%; 32/50 in controls; ncdr). 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, hepatocel., multifocal in ♂ (18%; 9/50 animals vs 2%; 1/50 in controls). ▪ ↑ Focus of altered cells-eosinophilic, hepatocel., focal or multifocal in ♂ (32%; 16/50 animals vs 6%; 3/50 in controls). *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).	Quast & McGuirk (1995) (CA) B.6.5-04

Type of study/data Species/strain No of animals	Test substance Route of exposure Dose levees, duration of exposure.	Observations	Reference
		500 mg/kg bw/day <u>Neoplastic changes</u> *Liver* ▪ ↑ hepatocellular adenoma in ♂ (80%; 40/50 animals vs 54%; 27/50 in controls). ▪ ↑ hepatocellular adenoma/ carcinoma/ hepatoblastoma in ♂ (90%; 45/50 animals vs 64%; 32/50 in controls; ncdr). <u>Non-neoplastic changes</u> *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, hepatocel., focal or multifocal in ♂ (24%; 12/50 animals vs 6%; 3/50 in controls). *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). **250 mg/kg bw/day** <u>Neoplastic changes</u> *Liver* ▪ ↑ Hepatocellular adenoma in ♂ (66%; 33/50 animals vs 54%; 27/50 in controls; n.s.). ▪ ↑ Hepatocellular adenoma/ carcinoma/ hepatoblastoma in ♂ (72%; 36/50 animals vs 64%; 32/50 in controls; ns; ncdr). <u>Non-neoplastic changes</u> *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). ▪ ↑ 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, hepatocel., focal or multifocal in ♂ (12%; 6/50 animals vs 6%; 3/50 in controls; ns). *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).	

Type of study/data Species/strain No of animals	Test substance Route of exposure Dose levees, duration of exposure.	Observations	Reference
		-Systemic LOAEL = 250 mg/kg bw/day. -Systemic NOAEL < 250 mg/kg bw/day. -Critical effect at the LOAEL: changes in hepatocytes and tubule morphology (♂,♀), ↓ bw/bwg (♀). -Neoplastic LOAEL= 500 mg/kg bw/day. -Neoplastic NOAEL= 250 mg/kg bw/day. -Critical effect at the LOAEL: ↑ incidence of hepatocellular adenoma (♂). **Target tissue/organ: Liver and kidney to a lesser extent.**	
Reproductive toxicity*			
Two-generation, rat CD Sprague-Dawley rats. Both sexes. At least 25/ Dose group. **Acceptable** *See table 57 for more information.*	OPP (purity 99.86%) Dietary 40, 140 and 490 mg/kg bw/day (Actual doses:35, 125, 457 mg/kg bw/day) for 2 generations.	*Only effects relevant for STOT RE* **Parental effects** **490 mg/kg bw/day** ***P:*** ▪ ↑ Rel. wt. 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%; ns) ▪ ↑ 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. ***F1:*** ▪ ↓ Abs. wt. of liver (13%) and kidney (9%) in ♀ ▪ ↑Rel. wt. 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 ♀ (ns; ndr) ▪ ↑ Average microns at 10X 62% in ♂ **140 mg/kg bw/day** ***P:*** ▪ ↑ Rel. wt. of ovaries in ♀ (19%, ns) ▪ ↑ Incidence of average no. cells/layer 29% in ♀. ↑ of average microns at 10X 48% in ♂ and 51% in ♀. ▪ ↑ Incidence of bladder calculi in ♂ (15/35 vs. 9/35 in controls (46% vs 26%; ns) ▪ ***F1:*** ▪ ↑ Abs. wt. 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:*** ▪ ↑ Rel. wt. of ovaries in ♀ (29%, ndr) ***F1:*** ▪ ↑ Abs. wt. of kidney (7%, ndr) and testes (6%, ndr) in ♂. **Offspring effects** **490 mg/kg bw/day**	Eigenberg (1990) (CA) B.6.6.1/01

Type of study/data Species/strain No of animals	Test substance Route of exposure Dose levees, duration of exposure.	Observations	Reference
		21 days or older *Kidney* *F1* ▪ ↑ Pelvis dilatation in F1a ♀ (100%, 8/8 animals vs 89%, 8/9 in controls; ns). ▪ ↑ Pelvis dilatation in F1b ♂ (25%, 1/4 animals vs 0% in controls; ns; ndr). *F2* ▪ ↑ Pelvis dilatation in F2a ♂ (33%, 5/15 animals vs 0%, in controls; ns; ndr). ▪ ↑ Pelvis dilatation in F2b ♂ (67%, 4/6 animals vs 25%, 1/4 in controls; ns; ndr). ▪ ↑ Pelvis dilatation in F2a ♀ (77%, 20/26 animals vs 50%, 4/8 animals, in controls; ns; ndr). ▪ ↑ Pelvis dilatation in F2b ♀ (80%, 4/5 animals vs 25%, 2/8 animals in controls; ns; ndr). **140 mg/kg bw/day** 21 days or older *Kidney* *F1* ▪ ↑ Pelvis dilatation in F1a ♀ (92%, 12/13 animals vs 89%, 8/9 in controls; ns.). **40 mg/kg bw/day** 21 days or older *Kidney* *F1* ▪ ↑ Pelvis dilatation in F1a ♀ (91%, 11/12 animals vs 89%, 8/9 in controls; n.s.). -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 to a lesser extent.**	
Two-generation, rat OECD 416. Deviations: Same as in the previous 2-generation study by Eigenberg (1990), except dams were cohoused for appropriate amounts of time. **Acceptable** Albino CD Sprague-Dawley rats. Both sexes. 30/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 ♂/♀).	*Only effects relevant for STOT RE* **Parental effects** **500 mg/kg bw/day** ***P:*** *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)]. **F1:** *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)	Eigenberg & Lake (1995) (CA) B.6.6.1-02

Type of study/data Species/strain No of animals	Test substance Route of exposure Dose levees, duration of exposure.	Observations	Reference
		Kidney: ▪ ↑ Incidence of kidneys debris in ♂ (4/30 vs. 0/30 in controls). ▪ ↑ Incidence of calculi in ♂ (7/30 vs 0/30 in controls). **-Target organ: Urinary bladder**	
Developmental toxicity, rabbit NZW Rabbit. Range finding study Females. 7 / Dose group. **Supportive only** *See table 60 for more information.*	OPP (purity 99.77%) Oral gavage **0, 250, 500 and 750 mg/ kg bw/day from day 7 to 19 of gestation.**	*Only effects relevant for STOT RE* **Maternal toxicity** **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. *Histopathology (No 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- increased, mucosa (43%, 3/7 animals vs 0% in controls). **500 mg/kg bw/day:** ***Gross pathology*** ▪ ↓ Bw gain [GD 7-10 (101%)]. ▪ ↑ Kidney abs./rel. wt (15%, ns/34%) ▪ Gross pathology: Pale kidneys. *Histopathology (No 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). **250 mg/kg bw/day:** ***Gross pathology*** ▪ ↑ kidney rel. wt (16%, ns). *Histopathology (No statistically analysed)* Kidney ▪ ↑ autolysis (14%, 1/7 animals vs 0% in controls). Liver	Zablotny *et al.* (1991b) (CA) B.6.6.2/03

Type of study/data Species/strain No of animals	Test substance Route of exposure Dose levees, duration of exposure.	Observations	Reference
		▪ ↑ 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. -Developmental LOAEL: cannot be stablished, since foetuses were not examined for skeletal, visceral and external anomalies.. -Developmental NOAEL: cannot be stablished, since foetuses were not examined for skeletal, visceral and external anomalies.. Critical effect at the LOAEL: - ***-Target tissue/organ : Kidney.***	
Developmental toxicity, rabbit NZW Rabbit. Females. 16 to 24 / Dose group. **Acceptable** *See table 60 for more information.*	OPP (purity 99.77%) Oral gavage 0, 25, 100, 250 mg/ kg bw/day from day 7 to 19 of gestation.	*Only effects relevant for STOT RE* **Maternal toxicity** **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 *Organ weights:* There was no effect of OPP treatment on the absolute or relative weights of liver and kidneys. *Histopathology (No 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. -Developmental LOAEL*: > 250 mg/kg bw/day. -Developmental NOAEL: ≥ 250 mg/kg bw/day.	Zablotny *et al.* (1991c) (CA) B.6.6.2/04

Type of study/data Species/strain No of animals	Test substance Route of exposure Dose levees, duration of exposure.	Observations	Reference
		Critical effect at the LOAEL: - **-Target tissue/organ: Kidney.**	
Other studies B.6.8.2 and B.6.8.3*			
Subchronic study into bladder effects Rats (CDF[F-344]/BR. Males. 20 / Dose group. **Supportive only** ***See table 55 for more information.***	OPP (purity 99.7%) Dietary 0, 1000, 4000 or 12,500 ppm (0, 54, 224, and 684 mg/kg bw/day) **for 13 weeks.**	*Only effects relevant for STOT RE* *Histopathology* **12500 ppm (684 mg/kg bw/day)** *Bladder* ▪ ↑ 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* ▪ ↑ 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). **4000 ppm (224 mg/kg bw/day)** ▪ ↑ 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.	Christenson *et al.* (1996a) (CA) B.6.8.2-02

Type of study/data Species/strain No of animals	Test substance Route of exposure Dose levees, duration of exposure.	Observations	Reference
		-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) (♂). **-Target tissue/organ: Kidney and bladder.**	
Subchronic study, ^{32}P-postlabeling Rats (CDF[F-344]/BR. Males. 22 / Dose group. *See table 55 for more information.*	OPP (purity 99.5%) Dietary 0, 800, 4000, 8000 or 12,500 ppm (0, 57, 285, 568, and 937 mg/kg bw/day) *for* 13 weeks.	*Only effects relevant for STOT RE* *Histopathology* **12500 ppm (937 mg/kg bw/day)** *Bladder* ▪ ↑ Rel wt (35%) ▪ ↑ Simple hyperplasia in 70% (7/10 animals vs 0% in controls) at week 13. *Kidney* ▪ ↑ Rel wt (18%). **8000 ppm (568 mg/kg bw/day)** *Bladder* ▪ ↑ Rel wt (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.).(a) ▪ ↑ Extensive necrosis and appearance of rounded cells in addition to polygonal cells (20% at week 13 vs 0% in controls; n.s.). (a) ▪ ↑ 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). (a) *Kidney* ▪ ↑ Rel wt (12%). (a) Electron microscopy analysis were only analysed in 8000ppm and control groups. -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.**	Christenson *et al.* (1996b) (CA) B.6.8.2-03
Pubertal development and thyroid function in intact juvenile/peripubertal female. Crl:CD(SD) rats. 15/dose. Acceptable *See table 55 for*	OPP (99.9% Purity) Oral gavage 50, 250, 900 mg/kg bw/day from PND 22 to 42.	*Only effects relevant for STOT RE* *Histopathology (No statistically analysed)* **900 mg/kg bw/day** *Kidney* ▪ ↑ 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;	Marty M.S *et al* (2012) (CA) B.6.8.3.8

Type of study/data Species/strain No of animals	Test substance Route of exposure Dose levees, duration of exposure.	Observations	Reference
more information.		multifocal (very slight) (14%, 2/14 animals vs 0% in controls). **250 mg/kg bw/day** *Kidney* ▪ ↑ Dilatation tubule; focal/multifocal (very slight or slight) (27%, 4/15 animals vs 13%, 2/15 animals in controls). **50 mg/kg bw/day** *Kidney* ▪ ↑ Dilatation tubule; focal/multifocal (very slight or slight) (20%, 3/15 animals vs 13%, 2/15 animals in controls). **-Target tissue/organ: Kidney.**	
Pubertal development and thyroid function in intact juvenile/peripubertal male. Crl:CD(SD) rats. 15/dose. *See table 55 for more information.*	OPP (99.9% Purity) Oral gavage 50, 250, 900 mg/kg bw/day from PND 23 to 53.	*Only effects relevant for STOT RE* *Histopathology (No statistically analysed)* **900 mg/kg bw/day** *Kidney* ▪ ↑ 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). ▪ Hyperplasia; epithelium; papilla; unilateral or bilateral; multifocal (very slight) (14%, 2/14 animals vs 0% in controls). **-Target tissue/organ: Kidney.**	Marty M.S *et al* (2012) (CA) B.6.8.3.9

2.6.3.1.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure (short-term and long-term toxicity)

Lanxess-Dow has presented 10 studies to assess the short-term toxicity of OPP (Table 46).

Of those ten initial studies, eight were oral and were conducted with rats (4), rabbits (1) or dogs (3); the remaining two studies were dermal and lasted 21-days and 4 weeks in rats and mice respectively.

All ten studies were reported over the period from 1945 to 1993. Three of them were GLP compliant, two were accepted, another was deemed as supportive but quite reliable, and seven were accepted only as additional information.

Oral studies in rat:

-Two 1-month dietary studies were presented; dose levels of 0, 2, 3, 4, 5 and 10% (0 to 100'000 ppm) and 0, 2, 20 and 200 mg/kg bw/day were tested in female and male rats respectively. None of them was accepted due to the guideline deviations, unsuitable methodology and very brief reports. Mortality from 30'000 ppm and slight growth retardation at 20'000 ppm were the only adverse effects observed in these studies.

-In a 3-month dietary and 6-month gavage study presented, dose levels of 0, 0.1, 0.3, 1 and 2% (0 to 20'000 ppm or 0 to 2000 mg/kg bw/day) and 0, 50, 100, 200 and 500 mg/kg bw/day were administered respectively. In

this study, the animals treated with OPP through the diet for 3 months, showed decreases in body weights at 20'000 ppm (2000 mg/kg bw/day) and increases in kidney, liver and spleen weights without histopathological changes from **10'000 ppm** (1000 mg/kg bw/day) on **(considered the NOAEL).** In the 6-months gavage study, slight increases in liver and kidney weights, also without histopathological changes, were seen in rats treated at **500 mg/kg bw/day;** so the **NOAEL** is stablished at **200 mg/kg bw/day**.

-In the 13-week dietary study, the animals received dose levels of 0, 1560, 3130, 6250, 12'500 and 25'000 ppm (98 to 1663 mg/kg bw/day). Kidneys and urinary bladder seem to be the target organs in males. Increases in relative liver weights without histopathological or clinical chemistry-related findings were seen from 3130 in males and at 12'500 ppm in females. From 6250 ppm in males and at 25'000 ppm in females, relative kidneys weights were increased, while at 12'500 ppm increased water consumption and occult blood in urine, indicative of kidney damage and increased relative bladder weights and abnormal growth in the bladder urothelium were seen in males. The increase in relative kidney weight at 6250 ppm, however, was less than 10% and should be considered non-adverse. A **NOAEL of 6250 ppm** corresponding to **761 mg/kg/day** was established based on increased relative bladder weights in males that might mark the onset of abnormal urothelial growth.

-Nephritic lesions, moreover necrosis and proliferative lesions of the urinary bladder (simple hyperplasia, papillary/nodular hyperplasia or papillomas) were described in 13-week dietary studies in rat assessed as mechanistic studies in Section B.6.8.

Oral studies in rabbit:

-In the 13-days oral study presented, the rabbits received by gavage dose levels of 0, 100, 500 and 1000 ppm. Target organs were not identified and only signs of general toxicity were observed. The **NOAEL** was established at **100 mg/kg bw/day** based on decreases in body weight and body weight gains and on increased in kidney weights at 500 mg/kg bw/day.

Dietary studies in dogs:

-In the 4-week gavage study, animals received dose levels of 0, 100, 200 and 300 mg/kg bw/day. Slight body weights decreases were seen in females treated at the high dose level. Repeated emesis was observed in both sexes from 200 mg/kg bw/day and occurred more frequently and involved greater volumes in the high-dose group than in dogs treated with 200mg/kg bw/day.

Regarding haematologic parameters, dose-related decreases in Hb and Hct values, together with RBC and platelets counts were seen in all treated males but unusually high values were observed for these parameters in one of the two control male dogs. Decreases in platelet counts were recorded in females treated at 200 and 300 mg/kg bw/day, although no dose-relationship was observed. However, all differences between mean values for treated and control group dogs were attributed to normal variability between animals, and all the data were within the normal historical control range of the laboratory (not provided), and/or their own pre-study range of values. The **NOAEL** was established at **100 mg/kg bw/day.**

-The one-year gavage study in which dose levels of 0, 30, 100 and 300 mg/kg bw/day were administered, was accepted as additional information due to an apparent unsuitable administration of the test substance that cause emesis in all dogs, treated and controls, during the in-life phase. In general, this effect occurred more frequently and involved greater volumes in the high-dose group than in dogs that received lower dosages

Regarding haematology, urinalysis and clinical chemistry parameters, no differences were found between treated groups and controls, except a decrease in the creatinine phosphokinase (CPK) levels that were noted in high dose male groups at study termination. RMS deems that this reduction is likely caused by low physical activity of animals in the ultimate phase of the study.

Two high dose male dogs died on test days 137 and 138, respectively. These animals had dark regions in the pulmonary parenchyma, with or without the presence of bloody fluid. These lesions were consistent with administration of test material into the lungs, resulting in anoxia/shock. There were no other OPP-related gross pathological findings.

On the other hand, there were no histopathological lesions attributable to OPP treatment. Dogs from all control and dose levels had a variety of minor inflammatory lesions in their lungs, trachea and larynx. The findings were interpreted as being secondary to the daily gastric intubation.

Microscopic evaluation of the two decedents confirmed that the inadvertent passage of the stomach tube and deposition of the test material in the lungs were the cause of death in the high dose male group, that died prior to termination of the study. The lungs of these dogs had pale eosinophilic material having variable sized clear

vacuoles in the lumen of most bronchi. Alveolar oedema was present in association with the test material within the lung.

A **NOAEL of 100 mg/kg bw/day** was established based on increased emesis resulting in lower body weight and food efficiency with respect to the controls at 300 mg/kg bw/day.

-In the one-year diet study, the dogs received dose levels of 0, 20, 200 and 500 mg/kg bw/day and only a slight increase in the kidneys weight was seen at the high dose level. No abnormalities in haematology or urinalysis analysis were noted at any of the tested levels. Moreover, no histopathological changes were recorded in any other organ or tissue in any treated dog. The **NOAEL** was considered **200 mg/kg bw/day**.

Dermal studies:

Two dermal studies (at 21 days in rat and at 4 weeks in mice) were presented.

-In the 21-day dermal study in rat, OPP was administered to the animals at dose levels of 0, 100, 500 and 1000 mg/kg bw/day by dermal occlusive application. No systemic toxicity was observed at any dose level and erythema and scaling of the skin at the application sites were seen in rats of both sexes treated from 500 mg/kg. These alterations were consistent with the diagnosis of local irritation. The systemic NOAEL was more than 1000 mg/kg bw/day and the dermal **NOAEL** was **100 mg/kg bw/day**.

-In the 4-week dermal study the mice were given dermal applications of OPP of 5.95, 11.4, 20.8, 35.7, or 55.5 mg per animal each in 0.1 mL acetone. No systemic toxicity was observed. Ulcerative lesions at the application sites were seen at all dose levels and females seem to be more sensitive than males. An estimated **LOAEL of 200 mg/kg bw/day (♂) and 240 mg/kg bw/day (♀)** was established.

From the summary of short-term studies presented, it can be concluded that the **urinary bladder** was identified as the **target organ for OPP in rats**; an **NOAEL of 761 mg/kg/day** (6250 ppm) was established.

In **rabbits** only signs of general toxicity were observed. The **NOAEL was established at 100 mg/kg bw/day** based on decreased bodyweight and bodyweight gains at 500 mg/kg bw/day

In dog, target organs were not identified. Non-adverse signs of general toxicity as non-significant decreases in body weights and food efficiency were seen in females. Emesis appeared only in the gavage studies and not in the diet study. This effect was observed in treated animals and controls but with a frequency and intensity dose-related. It can be concluded that the emesis could be related to the route of administration but a toxicological effect of OPP cannot be discarded. The **NOAEL in dog** was **100 mg/kg bw/day**.

The **overall oral short-term NOAEL** was **761 mg/kg bw/day** (13-week dietary study in rat).

In the short-term dermal studies, the only adverse effect observed was local skin irritation. The **dermal NOAEL was 100 mg/kg bw/day**.

Although only one short-term study from table 46 (B.6.3.3-01, Zempel & Szabo, 1993) was strictly accepted on the basis of GLP and OECD guideline compliance, there are other studies relevant to evaluate specific organ toxicity after repeated exposure in other sections (see table 48).

In rats, the target organ for long-term toxicity of OPP was the urinary bladder (Wahle & Christenson, 1996, B.6.5-02), where dose- and time dependent hyperplasias and neoplasias of the urinary bladder epithelium were found. The **overall NOAEL for oral long-term toxicity was 39 mg/kg** and it is based on structural alterations in the urinary bladder of male rats. In mice, the target organ for long-term toxicity of OPP was the liver (Quast & McGuirk, 1995, CA, B.6.5-04).

In the reproductive toxicity studies considered for STOT-RE, the target organ in rat was the urinary bladder once again (Eigenberg, 1990, B.6.6.1/01), while the NOAEL was 35 mg/kg and it is based on bladder calculi in male rats and urothelial hyperplasia in males and females.

Ortho-Phenylphenol classification and labelling is listed in **Annex VI** of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). **Classification regarding specific target organ toxicity (repeated exposure) is not included**.

There are no repeated-dose toxicity studies with **SOPP**. Both SOPP and OPP are considered to be toxicologically equivalent under the conditions of such a studies, as suggested by Sato *et al.* (1988, B.6.1.1-01) and Reitz *et al.* (1983, B.6.1.1-03) (table 48), who show an essentially identical toxicokinetic behaviour and metabolite producing pattern.

Table 49: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/day)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure (mg/kg bw/day)	Classification supported by the study
Hodge *et al*. (1952). B.6.3.2-01	500	5 d/w, 6 months	500x2x(5day/7days)≈714	None
Cosse *et al.* (1990). B.6.3.2-03	300	52-weeks.	300x4 = 1200	None
Hodge *et al*. (1952). B.6.3.2-04	500	1-year	500x4 = 2000	None
Zempel & Szabo (1993). B.6.3.3-01	> 1000	21-days	-	None
Eigenberg (1990). B.6.6.1-01	125	50 to 70 weeks	125x50wk/13wk≈480	None
Wahle & Christenson (1996). B.6.5-02	200	2-years	200x104wk/13wk=1600	None
Quast & McGuirk (1995). B.6.5-04	250	2-years	250x104wk/13wk=2000	None

2.6.3.1.2 Comparison with the CLP criteria regarding STOT RE (specific target organ toxicity-repeated exposure)

Substances are classified as specific target organ toxicants following repeated exposure by the use of expert judgement, on the basis of the weight of all evidence available, including the use of recommended guidance values which take into account the duration of exposure and the dose/concentration which produced the effect(s), (see table below). Based on this, substances are placed in two distinct categories:

Category 1. Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below, to be used as part of a weight-of evidence evaluation.

Category 2. Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure.

Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below.

In exceptional cases human evidence can also be used to place a substance in Category 2.

Guidance values to assist in STOT RE classification

Categories	Route of exposure	Guidance values (dose/ concentration) for 90-day studies
Category 1	Oral (rat)	C ≤ 10 mg/kg bw/d
	Dermal (rat or rabbit)	C ≤ 20 mg/kg bw/d
Category 2	Oral (rat)	10 < C ≤ 100 mg/kg bw/d
	Dermal (rat or rabbit)	20 < C ≤ 200 mg/kg bw/d

All available evidence, and relevance to human health, shall be taken into consideration in the classification process, including but not limited to the following toxic effects in humans and/or animals:

(a) morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance

or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites;

(b) significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g. sight, hearing and sense of smell)

(c) any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters;

(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination;

(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity;

(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver);

(g) evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration.

The **urinary bladder** was identified as **target organ in male rats**, suggested by increased bladder weight and urothelial hyperplasia. This effect constitutes "significant organ damage", even though it did not significantly affect long-term survival in the 2-year rat study (Wahle, B.S. and Christenson, W.R., 1996, B6.5/02).

The **extrapolated (to 90-day exposure) effective doses** at which significant toxic effects (of relevance to human health) occur (after repeated exposure to OPP) are all above guidance values for classification as STOT RE category 2 listed (see table 49). Not a single repeated exposure study contains an effective dose (LOAEL) that, when extrapolated to 90 days would be low enough to consider classification in a STOT-RE category.

Strictly speaking, the **effective dose** would lie somewhere between LOAEL and NOAEL. However, even the NOAELs for these effects are higher than the guidance values for classification as STOT RE category 2. The lowest relevant NOAELs are 761 and 39 mg/kg bw/day mg/kg, in the 90-day oral rat study (5.3.2/02; Iguchi *et al.*, 1984) and the 2-year rat study (5.5/02; Wahle & Christenson, 1996), respectively. The 2-year NOAEL needs to be adjusted using Haber's law to be comparable with the guidance values. Thus the **adjusted NOAEL** from the 2-year study is (104 wk/13 wk) × 39 mg/kg bw/d = **312 mg/kg bw/day**.

Both NOAELs **are higher than the guidance** values for either STOT-RE category.

2.6.3.1.3 Conclusion on classification and labelling for STOT RE (specific target organ toxicity-repeated exposure)

Based on the data available for ***ortho*-phenylphenol (OPP)**, and according to the criteria under Regulation (EC) No. 1272/2008, RMS proposes no classification for this active substance in this hazard class (STOT-RE).

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</td><td>There were no reported adverse effects attributable to OPP administration</td><td>Macintosh, 1945</td></tr>
</table>

No guideline 15 White male rats/dose OPP Oral gavage: 0, 2, 20, 200 mg/kg bw/day, for 32-days	LOAEL > 200 mg/kg bw/day NOAEL = 200 mg/kg bw/day **Target organs/tissues were not identified**	B.6.3.1-02
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 histopathology not conducted 2 NZW female rabbits /dose OPP (99.77% purity) Oral gavage: 0, 100, 500 or 1000 mg/kg bw/day	<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> • ↓ 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.	B.6.3.1-03, 1991a
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	<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*	B.6.3.1-04, 1990

300 due to emesis) mg/kg bw/day 5 days a week for four weeks	• ↓ 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	
3-month dietary No guideline Rats of unspecified strain. 12 rats/sex/dose OPP (≥ 98% purity) Dietary: 0, 100, 300, 1000 and 2000 mg/kg bw/day	<u>2000 mg/kg bw/day</u> • Slight growth retardation (no detailed data provided in the study) • ↑ 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	Hodge *et al.*, 1952 B.6.3.2-01
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.	<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>	Iguchi *et al.*, 1984 B.6.3.2-02

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	*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%) *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%) Histopathology • Abnormal growth in the bladder mucosa in ♂ ♂/♀ (761/ 803 mg/kg bw/day) • Liver: ↑ relative weight in ♂ (7%) • Thymus: ↓ relative weight in ♂ (13%, ndr) and ↑ relative weight in ♀ (10%, ndr) • Kidney: ↑ relative weight in ♂ (4%) ♂/♀ (391/411 mg/kg bw/day) • 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.	
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	<u>Mortality</u>: 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 <u>500 mg/kg bw/day</u> *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 5 Fischer 344 rats/sex/dose 5 days/week for 21-days 0, 100, 500 and 1000 mg/kg bw/day	<u>1000 mg/kg bw/day</u> *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) <u>500 mg/kg bw/day</u> *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**	B.6.3.3-01, 1993
4-week	Ulcerative lesions at the site of application were observed in all mice that received ≤ 20.8 mg OPP; in 6/10 males and	National Toxicology

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	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	Program (NTP), 198 B.6.3.3-02
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)	B.6.5-02, 1996

	• ↑ 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 (♂) **Target tissue/organ: urinary bladder**	
Dietary in mouse 50 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)	B.6.5-04, 1995

- ↑ 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)

500 mg/kg bw/day

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)

250 mg/kg bw/day

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)
- ↑ 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	Parental effects *490 mg/kg bw/day: P generation*	B.6.6.1/01, 1990

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	• ↑ 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) *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	<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	B.6.6.1-02, 1995

doses: 18/17, 93/92, 459/457 mg/kg bw/day for ♂/♀)	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**	
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-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)*	B.6.6.2/03, 1991b

	• 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 **Target tissue/organ: kidney**	B.6.6.2/04, 1991c
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)	B.6.8.2-02, 1996a

	• ↑ 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) (♂) **Target tissue/organ: kidney and bladder**	
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	B.6.8.2-03, 1996b

	NOAEL = 285 mg/kg bw/day Critical effect at the LOAEL: ↑ of mitotic activity and hyperplasia of the urothelium **Target tissue/organ: bladder**	
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	900 mg/kg bw/day: kidney histopathology (not statistically analysed) • ↑ 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) 250 mg/kg bw/day: kidney histopathology (not statistically analysed) • ↑ Dilatation tubule; focal/multifocal (very slight or slight) (27%, 4/15 animals vs 13%, 2/15 animals in controls) 50 mg/kg bw/day: kidney histopathology (not statistically analysed) • ↑ 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 OPP (99.9% purity) Oral gavage: 50, 250, 900 mg/kg bw/day from PND 23 to 53	900 mg/kg bw/day: kidney histopathology (not statistically analysed) • ↑ 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) • Hyperplasia; epithelium; papilla; unilateral or bilateral; multifocal (very slight) (14%, 2/14 animals vs 0% in controls) **Target tissue/organ: kidney**	B.6.8.3.9, 2012

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.**

2.6.4 Summary of genotoxicity / germ cell mutagenicity [equivalent to section 10.8 of the CLH report template]

Table 50: Summary table of genotoxicity/germ cell mutagenicity tests *in vitro*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Bacterial gene mutation (Ames test) Comparable to OECD TG 471 (1983) Deviations from the current OECD TG 471 (2020):	*ortho*-Phenylphenol Purity not stated Vehicle: acetone *S. typhimurium:* TA98, TA100, TA1535, TA1537,	Preliminary study in TA100 ± S9 (hamster) showed cytotoxicity at the highest dose. The dose range selected were 667 (-S9) and 1000 (+S9) µg/plate.	**Negative** ± S9 Toxicity at the high dose levels tested in TA98 and TA100 in the first experiment.	San, R. H. C. and Springfield, K. A. (1989a) (CA) B.6.4.1.1-01

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Characterisation and stability of test item not determined. TA102 or *E.Coli* WP2 uvrA not tested. GLP: Yes **Study acceptable**	TA1538 Rat and Hamster S9			
Bacterial gene mutation (Ames test) Deviations from the current OECD TG 471 (2020): Characterisation and stability of test item not determined, only 4 strains used, data on concentration range or positive controls not reported GLP: No **Supporting information**	*ortho*-Phenylphenol Purity not stated Vehicle: DMSO *S. typhimurium:* TA97, TA98, TA100 and TA102 Rat S9	No information on test concentrations and no result table available.	**Negative** ± S9	Pagano, G. *et al* (1988) (CA) B.6.4.1.1-02
Bacterial gene mutation (Ames test) Pre-guideline Deviations from the current OECD TG 471 (2020): Characterisation and stability of test item not determined, data on test concentration range, positive controls not reported GLP: No **Supporting information**	*ortho*-Phenylphenol Purity not stated Vehicle not stated *S. typhimurium:* TA98, TA100, TA1535, TA1537 and TA1538 *E. coli* WP2 *hcr* S9	No information on test concentrations and no result table available.	**Negative** ± S9	Shirasu, Y *et al* (1978a) (CA) B.6.4.1.1-03
Bacterial gene mutation (Ames test) Comparable to OECD TG 471 (1997) Deviations from the current OECD TG 471 (2020): only 4 strains used. GLP: No	*ortho*-Phenylphenol Purity: 99.9% Lot No.: MM09157 Vehicle: DMSO *S. typhimurium:* TA98, TA100, TA1535 and TA1537	Dose-range study with TA100 ±S9 up to 10000 µg/plate. 3.3 – 200 µg/plate	**Positive in TA1535 –S9** Slight positive increase in revertants at 100 µg/plate	Haworth, S. *et al*, 1983 (AS) B.6.4.1.1-04

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Study acceptable	Rat and Hamster S9			
Bacterial gene mutation (Ames test) Pre-guideline Deviations from the current OECD TG 471 (1997): Characterisation and stability of test item not determined, data on test concentration range, positive/negative controls not reported GLP: No **Supporting information**	*ortho*-Phenylphenol Purity not stated Vehicle not stated *S. typhimurium:* TA98, TA100 S9	No information	**Weakly positive in TA98** ± S9	Nishioka, H. and Ogasawara, H. (1978) (CA) B.6.4.1.1-05
Bacterial gene mutation (Ames test) Deviations from the current OECD TG 471 (2020): Characterisation and stability of test item not determined, data on test concentration range, positive controls not reported GLP: No **Supporting information**	*ortho*-Phenylphenol Purity not stated Vehicle: not reported *S. typhimurium:* TA98, TA100, TA1535, TA1537 and TA1538 *E. coli* WP2 *hcr* S9	No information on test concentrations and no result table available.	**Negative** ± S9	Moriya, M. *et al* (1983) (CA) B.6.4.1.1-06
Bacterial gene mutation (modified Ames test) Pre-guideline Deviations from the current OECD TG 471 (2020): Characterisation and stability of test item not determined, results data not reported, positive/negative controls not reported GLP: No **Supporting information**	*ortho*-Phenylphenol Purity not stated Vehicle not stated *S. typhimurium*: G46, TA1535, C3076, TA100, TA1537, D3052, TA1538 and TA98 *E. coli* WP2 and WP2 *uvrA* Rat S9	No information on test concentrations and no result table available.	**Negative** ± S9	Probst, G. S. *et al* (1981) (CA) B.6.4.1.1-07

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Bacterial gene mutation (modified Ames test) Pre-guideline Deviations from the current OECD TG 471 (2020): Characterisation and stability of test item not determined, results not reported GLP: No **Supporting information**	*ortho*-Phenylphenol Purity not stated Vehicle not stated *S. typhimurium*: G46, TA1535, C3076, TA100, TA1537, D3052, TA1538 and TA98 *E. coli* WP2 and WP2 *uvrA* Rat S9	No information on test concentrations and no result table available.	**Negative ± S9**	McMahon R. E. *et al* (1979) (CA) B.6.4.1.1-08
Bacterial gene mutation (modified Ames test) Pre-guideline Deviations from the current OECD TG 471 (2020): Characterisation and stability of test item not determined, results not reported GLP: No **Supporting information**	*ortho*-Phenylphenol Purity not stated Vehicle not stated *S. typhimurium*: G46, TA1535, C3076, TA100, TA1537, D3052, TA1538 and TA98 *E. coli* WP2 and WP2 *uvrA* Rat S9	No information on test concentrations and no result table available.	**Negative ± S9**	Cline, J. C. and McMahon R. E. (1977) (CA) B.6.4.1.1-09
Bacterial gene mutation (Ames test) Deviations from the current OECD TG 471 (2020): Purity of test substance not reported GLP: Not stated **Study acceptable**	*o*-Phenylphenol, sodium salt tetrahydrate *S. typhimurium:* TA98, TA100, TA1535, TA1537 and TA1538	Plate incorporation 3.3 – 3333 µg/plate	**Negative ± S9**	San, R. H. C. and Springfield, K. A. (1989b) (CA) B.6.4.1.1-10
Induction of ouabain resistance in human RSa cells No guideline GLP: No **Supporting information**	*ortho*-Phenylphenol Purity not stated Vehicle: ethanol RSa (human cell strain)	Dose: 0-30 µg/mL	**Dose-related increase in the frequency of ouabain-resistant mutants** **Positive**	Suzuki, H. *et al* (1985) (CA) B.6.4.1.2-01

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
HGPRT forward mutation assay GLP: Yes **Study acceptable**	*ortho*-Phenylphenol Purity: 99.9 % CHO-WB1 cells	6.25 – 100 µg/mL (-S9) 12.5 – 115 µg/mL (+S9)	**Negative ± S9** High cytotoxicity observed at high dose levels tested with and without metabolic activation.	Brendler, S. (1992) (CA) B.6.4.1.2-02
TK+/- mutation assay in L5178Y cells (mouse lymphoma assay) Deviations from current OECD TG 490 (2016): Characterisation of test item not determined (purity), poor description of method (duration of exposure to test item, cell line origin) historical control data not provided GLP: Yes **Study acceptable**	*ortho*-Phenylphenol Purity not stated L5178Y TK $^{+/-}$ Solvent: ethanol	18 – 44 µg/mL (-S9) 5 – 31 µg/mL (+S9) Preliminary cytotoxicity test indicated that doses above 50 µg/mL (up to 2000 µg/mL) were highly cytotoxic	**Negative ± S9** The mutant frequency exceeded the global evaluation factor (GEF) at doses with less than 10 % total growth, hence the positive response observed +S9 is regarded as negative.	Harbell, J. W., 1989 (CA) B.6.4.1.2-03
TK+/- mutation assay in L5178Y cells (mouse lymphoma assay) Deviations from current OECD TG 490 (2016): historical control data not provided GLP: No **Study acceptable**	*ortho*-Phenylphenol Purity > 99 % L5178Y TK $^{+/-}$ Solvent: water (-S9), DMSO (+S9)	20 – 60 µg/mL (-S9) 0.32 – 5 µg/mL (+S9)	**Negative +S9** The mutant frequency exceeded the global evaluation factor (GEF) at doses with less than 10 % total growth, hence the positive response observed +S9 is regarded as negative.	NTP (1986) (CA) B.6.4.1.2-04
Mammalian cell chromosome aberration test Deviations from current OECD TG 473 (2016): No detailed experimental results data reported, only 100 metaphases scored, no metabolic activation, gaps not evaluated, no historical control data available GLP: No	*ortho*-Phenylphenol Purity not stated Chinese hamster lung fibroblasts (CHL) Solvent: DMSO No metabolic activation used	Up to 0.05 mg/mL 48 h expression time No metabolic activation used	**Negative -S9**	Ishidate, M. *et al* (1984) (CA) B.6.4.1.3-01

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Supporting information				
Mammalian cell chromosome aberration test Deviations from current OECD TG 473 (2016): Only 200 metaphases scored Gaps included in the chromosome aberration result, no historical control data and positive control data provided, no metabolic activation used. GLP: No **Supporting information**	*ortho*-Phenylphenol Purity > 99 % Chinese hamster ovary (CHO-K1) Solvent: DMSO No metabolic activation used	Dose: 50-175 µg/mL IC50 27 h and 42 h expression time	**Positive -S9 for Sister chromatid exchanges at 27 h expression time** **Positive chromosome aberration -S9 both at 27 h and 42 h expression time**	Tayama-Nawai, S. *et al* (1984) (CA) B.6.4.1.3-02
Mammalian cell chromosome aberration test Deviations from current OECD TG 473 (2016): Only 100 metaphases scored, no method described (only summary results table provided), no metabolic activation, no historical control data GLP: No **Supporting information**	*ortho*-Phenylphenol (OPP) *ortho*-Phenylphenol sodium salt (SOPP) Chinese hamster lung fibroblasts (CHL-1-147) Solvent: DMSO (OPP) Saline (SOPP) No metabolic activation used	24 h and 48 h Collection of cytogenetic data from publications	**OPP: Negative -S9** **SOPP: Negative -S9**	Ishidate, M. (1983) (CA) B.6.4.1.3-03
Mammalian cell chromosome aberration test Compilation of results for OPP and SOPP GLP: No **Supporting information**	*ortho*-Phenylphenol (OPP) Purity not stated *ortho*-Phenylphenol sodium salt (SOPP) Purity not stated Solvent: DMSO Chinese hamster lung fibroblasts (CHL)	Compilation of experimental results from publications	**OPP:** **Positive -S9 CHO-K1 cells, 100 µg/mL, 3 h treatment** **Negative -S9, CHL** **SOPP:** **Positive, -S9, CHO-K1, 50 µg/mL** **Negative, -S9 CHL**	Ishidate, M. Jr *et al* (1988) (CA) B.6.4.1.3-04

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Mammalian cell chromosome aberration test Deviations from current OECD TG 473 (2016): Only 100 metaphases scored, no experiments without S9 mix, no historical control data GLP: No **Study acceptable**	*ortho*-Phenylphenol 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, S. *et al* (1989) (CA) 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 **Study acceptable**	*ortho*-Phenylphenol 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)	**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, S. and Nakagawa, Y. (1991) (CA) B.6.4.1.3-06
DNA single strand breaks and 8-OH-dG formation No guideline GLP: No **Supporting information**	*ortho*-Phenylphenol Phenylhydroquinone Phenylbenzoquinone Purity not stated Chinese hamster V79 lung fibroblasts	OPP: 50-400 µM PHQ: 25-45 µM PBQ: 20-30 µM	**OPP itself did not cause DNA single strand breaks or 8-OH-dG formation.** **The metabolites PHQ and PBQ caused a significant increase in both parameters at non-cytotoxic concentrations**	Henschke, P. *et al* (2000) (CA) B.6.4.1.4-01
Bacterial DNA repair assay GLP:No **Supporting information**	*o*-Phenylphenol Purity not stated Rec-assay *B. subtilis* H17 and M45	No dose information	**Negative in all assays**	Shirasu, Y. *et al* (1978b) (CA) B.6.4.1.4-02
Bacterial DNA repair assay GLP:No **Supporting information**	*o*-Phenylphenol Purity not stated *E.coli* WP2, WP2 *uvrA*, CM571 and WP100	No dose information	**Positive in DNA repair tests**	Nishioka, H. and Ogasawara, H. (1978) (CA) B.6.4.1.4-03

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
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 **Supporting information**	*ortho*-Phenylphenol Purity not stated Rat F344 hepatocytes	100 nmol/mL	**Negative in UDS assay *in vitro***	Probst, G. S. *et al* (1981) (CA) B.6.4.1.4-04
DNA reactivity in the presence of Copper (II) ions No guidance GLP: No **Supporting information**	*ortho*-Phenylphenol Phenylbenzoquinone Phenylhydroquinone Purity not stated ^{32}P-5'-End labeled DNA fragments from plasmid pbcNI		**PHQ and PBQ plus H_2O_2 caused strong DNA damage**	Inoue, S. *et al* (1990) (CA) B.6.4.1.4-05
DNA reactivity No guidance GLP: No **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.** **OPP and PBQ display no similar reactivity.**	Nagai, F. *et al* (1990) (CA) B.6.4.1.4-06
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 $CuCl_2$ and $FeCl_2$ 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, F. *et al* (1995) (CA) B.6.4.1.4-07
In vitro comet assay No guidance GLP: No	*ortho*-Phenylphenol Purity: 99 %	Concentration 0-800 µM	**Significant increase of DNA strand breaks at 400 and 800 µM** **Hydroxytyrosol**	Li, J. *et al* (2012) (CA) B.6.4.1.4-08

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Supporting information				

Table 51: Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Chromosome aberration *in vivo* Pre-guidance Deviations from OECD TG 475 (2016): purity not stated, no vehicle information, method poorly described (abstract), no individual data reported, no pos/neg control or historical control data reported GLP: No **Supporting information**	*ortho*-Phenylphenol Purity not stated Male Wistar rats Bone marrow cells	Oral daily doses of 50, 100, 200, 400 and 800 mg/kg for 5 days Single doses of 250, 500, 1000, 2000 and 4000 mg/kg Animals were killed 24 h after treatment	**Negative in any tested doses (single or repeat exposure)**	Shirasu, Y. *et al* (1978a) (CA) B.6.4.2.1-01
Chromosome aberration *in vivo* Pre-guidance Deviations from OECD TG 475 (2016): purity not stated, no positive controls used, experimental method poorly described (no. of animals, slide preparation), no historical control data reported. GLP: No **Supporting information**	*ortho*-Phenylphenol sodium salt (SOPP or OPP-Na) Purity not stated Male JCL-ICR mice Male F344/Du rats Bone marrow cells	<u>Mice</u>: Oral gavage Doses: 0, 300, 600 and 1200 mg/kg Volume: 10 mL Vehicle: distilled water Exposure duration: 6, 24 and 48 h. Animals were killed 24 h after treatment <u>Rats</u>: Oral diet Doses: 1, 2 or 4 % OPP-Na Exposure duration: 13 weeks	**Negative** **No chromosomal aberrations identified in murine bone marrow cells in mice and rats**	Yoshida, S. *et al* (1979) (CA) B.6.4.2.1-02
Comet assay *in vivo* Pre-guidance Deviations from OECD TG 489 (2016): 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 historical control data reported GLP: Yes **Supporting information**	Preventol o-extra (OPP) Purity: 99.8 % Male CD-1 mice Liver and kidney	Oral gavage Doses: 0, 250, 2000 mg/kg Volume: 10 mL Vehicle: olive oil 4 mice/group Exposure duration: 3, 8 and 24 h. Animals were killed after treatment (3, 8 and 24 h)	**Negative** **No increases in tail-length in hepatocytes and kidney cells** **Two animals died in the top dose group (2000 mg/kg)**	Brendler-Schwaab, S. (2000) (CA) B.6.4.2.2-01
Comet assay *in vivo*	OPP	Dose: 0, 2000 mg/kg	**OPP induced DNA**	Sasaki, Y. F.

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Pre-guidance Deviations from OECD TG 489 (2016): 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 GLP: No **Supporting information**	Purity not stated Male CD-1 mice Liver, lung, kidney, spleen, brain, bladder and bone marrow	Volume: 10 mL Vehicle: 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**	*et al* (1997) (CA) B.6.4.2.2-02
Comet assay *in vivo* Pre-guidance Deviations from OECD TG 489 (2016): purity of test item not reported, number of total cells per organ is less than 150, body weight not recorded at the start and at the end of the experiment GLP: No **Study acceptable**	OPP sodium salt tetrahydrate Male Sprague-Dawley rats Liver and stomach	Dose: 0, 250, 500 and 1000 mg/kg Volume: 10 mL 5 animals/group Exposure duration: 3 days Animals were sacrificed 3 h after the last dose administration Vehicle: corn oil	**OPP-Na did not induce DNA strand breaks or nuclei in liver or stomach cells**	De Boeck, M. *et al* (2015) (CA) B.6.4.2.2-03
DNA alkaline elution assay *in vivo* No guideline GLP: No **Supporting information**	*ortho*-Phenylphenol (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 Vehicle: 0.9 % NaCl solution Intravesical injection into the bladder Exposure: 10 min	**OPP and PHQ: Negative** **PBQ Positive**	Morimoto, K. *et al* (1987) (CA) B.6.4.2.3-01
DNA alkaline elution assay *in vivo* No guideline GLP: No **Supporting information**	*ortho*-Phenylphenol (OPP) Purity: 98 % *ortho*-Phenylphenol sodium salt (OPP-Na) Purity not stated	<u>*In situ* study</u>: OPP Dose: 0.05 % PHQ Dose: 0.05 % PBQ Doses: 0.0005-0.1 % Volume: 0.4 mL	**PBQ caused DNA damage in the urinary bladder epithelium.** **OPP and PHQ did not cause DNA damage in the**	Morimoto, K. *et al* (1989) (CA) B.6.4.2.3-02

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
	Phenylhydroquinone (PHQ) Purity: 99 % Phenylbenzoquinone (PBQ) Purity: > 99 % Male F344/DuCrj rats Urinary bladder epithelium	Vehicle: 0.9 % NaCl solution Intravesical injection into the bladder Exposure: 10 min <u>Feeding study</u>: OPP-Na Dose: 0.5, 1.0 and 2.0 % in the diet 5-10 animals/dose group Duration of exposure: 3-5 months	**bladder epithelium** **Repeat exposure to OPP-Na in the diet during 3-5 months caused DNA damage in the urinary bladder epithelium.**	
In vivo study for ploidy No guideline GLP: No **Supporting information**	*ortho*-Phenylphenol (OPP) Purity not stated Urinary bladder epithelial cells	Doses: 800, 2000, 4000, 8000 and 12500 ppm Oral diet Duration of exposure: 14 days	**OPP did not cause hyperploidy or ploploidy in proliferating bladder epithelial cells**	Balakrishnan, S. and Eastmond, D.A. (2003) (CA) B.6.4.2.3-03
UDS *in vivo* Pre-guidance Deviations from OECD TG 486 (1997): urinary bladder epithelial cells are not the subject of the guideline, purity of the test substance not reported, only one dose studied GLP: No **Supporting information**	*ortho*-Phenylphenol sodium salt (OPP-Na) Purity not stated Female rats BOR:WISW Urinary epithelial cells	Dose: 100 mg/kg bw Oral gavage Vehicle: alkaline solution Volume: 10 mL Duration of exposure: Experiment A: 24 h Experiment B: 7 days	**OPP-Na induced UDS in urinary bladder epithelial cells**	Klein, W. (1986) (CA) B.6.4.2.3-04
Dominant Lethal test Pre-guidance Deviations from current OECD TG 478 (2016): 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 historical control data reported. GLP: No **Supporting information**	*ortho*-Phenylphenol Purity: 99.7 % C3H Male mice	Dose: 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	**Negative**	Kaneda, M. *et al* (1978) (CA) B.6.4.3.1-01

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Dominant Lethal test Pre-guidance Deviations from current OECD TG 478 (2016): only abstract provided, no adequate study description GLP: No **Supporting information**	*ortho*-Phenylphenol (OPP) Purity not stated C3H Male mice	Dose: 0, 100 and 500 mg/kg bw Oral Duration of exposure: 5 days	**Negative**	Shirasu, Y. *et al* (1978a) (CA) B.6.4.3.1-02
Sex-linked recessive lethal test in *Drosophila* No guideline stated Deviations from OECD TG 477 (1984): 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 **Supporting information**	*ortho*-Phenylphenol (OPP) Purity: 99 % Male and Female *Drosophila*	Dose: 250 ppm in feed or 500 ppm by injection Vehicle: 5 % sucrose solution	**Negative**	NTP (1986) (CA) B.6.4.3.1-03

Table 52: Summary table of human data relevant for genotoxicity / germ cell mutagenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity / germ cell mutagenicity

Most data to address this point were presented in the original DAR (2008) in support of the inclusion of *ortho*-phenylphenol in Annex I of Directive 91/414/EEC and were deemed acceptable following evaluation and peer review at EU level. A total of five new genotoxicity studies have been submitted for the renewal process to address the genotoxicity of *ortho*-phenylphenol (*in vitro* Comet assay) and *ortho*-phenylphenol sodium salt (Ames test and *in vivo* UDS, chromosome aberration and Comet assays). All data presented for the renewal evaluation of the active substance has been reviewed and evaluated.

***ortho*-Phenylphenol**

A total of 36 studies have been submitted for the renewal process to evaluate the genotoxicity of *ortho*-phenylphenol, of which a total of 27 correspond to studies *in vitro* and 9 correspond to studies *in vivo*. Only one new study (*in vitro* Comet assay) has been submitted and the remaining studies were presented and evaluated as part of the original DAR (2008).

In Commission Regulation (EU) No. 283/2013 *in vitro* photomutagenicity studies may be indicated by the structure of a molecule. If the ultraviolet/visible (UV/VIS) molar extinction/absorption coefficient of the active substance and its major metabolites is less than 1000 L x mol $^{-1}$ x cm $^{-1}$ photomutagenicity testing is not required. In the case of *ortho*-phenylphenol (OPP) the molar extinction/absorption coefficient is 8200 L x mol^{-1} x cm^{-1} at a

maximum absorbance of 287 nm (Erstling, K., 2004). Whilst photomutagenicity testing is potentially triggered, the *in vitro* 3T3 NRU phototoxicity assay returned a negative result (Leuschner, 2018, B.6.2.7) and thus no photomutagenicity testing is considered to be necessary.

***In vitro studies*:**
OPP showed negative results *S.typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 in the presence and absence of metabolic activationin (San and Springfied, 1989a, B.6.4.1.1-01). This study did not include a strain to test for cross-linking mutagens. OPP was reported negative in *S. typhimurium* TA102 (Pagano *et al*, 1988, B.6.4.1.1-02) and *E. coli* WP2 in a number of studies although they were considered as supporting information based on method deficiencies. OPP gave a slight positive increase in revertants in TA1535 without metabolic activation (Haworth *et al*, 1983, B.6.4.1.1-04) but this result has not been reproduced in any of the additional supporting information studies. Based on the available information, it can be concluded that OPP is not mutagenic in bacteria gene mutation assays.

OPP did not cause gene mutations in the HGPRT forward mutation assay in CHO-WB1 cells in the presence or absence of metabolic activation (Brendler S., 1992, B.6.4.1.2-02). OPP was concluded negative in two mouse lymphoma assays (Harbell, 1989, B.6.4.1.2-03; NTP, 1986, 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 would not be considered positive, and therefore, the overall results is considered to be negative. Based on the data available, OPP is not 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 (Tayama *et al*, 1989, B.6.4.1.3-05; Tayama and Nakagawa, 1991, B.6.4.1.3-06). OPP also produced sister chromatid exchanges in the presence of metabolic activation. Both studies are not GLP compliance and therefore, are deemed reliable supporting information. OPP was reported negative in the absence of metabolic activation in CHL cells (Ishidate *et al*, 1984, B.6.4.1.3-01; Ishidate M., 1983, B.6.4.1.3-03) or CHO-K1 cells (Tayama.Naway *et al*, 1984, B.6.4.1.3-02; Ishidate *et al*, 19898, B.6.4.1.3-04) although based on methodology deficiencies these studies are considered supporting information only. The metabolites phenylhydroquinone (PHQ) and phenylbenzoquinone (PBQ) also produced chromosome aberrations in CHO-K1 cells in the presence and absence of metabolic activation (Tayama and Nakagawa, 1991, B.6.4.1.3-06). Based on the data available, OPP induces chromosomal aberration and SCE's *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 (Probst *et al*, 1981, B.6.4.1.4-02). 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 (Li *et al*, 2012, B.6.4.1.4-06).
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 (Henschke *et al*, 2000, B.6.4.1.4-01; Nagai *et al*, 1995, B.6.4.1.4-05). A number of studies reported the DNA damage caused by PHQ and PBQ but not OPP (Inoue *et al*, 1990, B.6.4.1.4-03; Nagai *et al*, 1990, B.6.4.1.4-04), all of them regarded as supporting information.

***In vivo*:**
OPP was negative in a cytogenetic study in bone marrow cells of rats (Shirasu *et al*, 1978a, B.6.4.2.1-01). However, this study can only be considered as supporting information (abstract).

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 (Brendler-Schwaab, 2000, 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 (Sasaki *et al*, 1997, B.6.4.2.2-02). The former study follows the method described in the latter. 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* (Morimoto *et al*, 1987, B.6.4.2.3-01; Morimoto *et al*, 1989, 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 (Balakrishnan and Eastmond, 2003, B.6.4.2.3-03).

Three *in vivo* germ cell studies were submitted and evaluated at EU level in the previous DAR (2008). In the dominant lethal tests, OPP gave a negative result (Kaneda *et al*, 1978, B.6.4.3.1-01; Shirasu *et al*, 1978, B.6.4.3.1-02). OPP was also negative in a sex-linked recessive lethal test in *Drosophila* (NTP, 1986, B.6.4.3.1-03). These studies were not performed under GLP and one is an abstract of a peer-reviewed publication, hence they are considered as supporting information.

ortho-Phenylphenol classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). No classification for germ cell mutagenicity is included.

Based on all the available genotoxicity data, the induction of chromosomal aberrations *in vitro* in mammalian cells (Tayama *et al*, 1989, B.6.4.1.3-05; Tayama and Nakagawa, 1991, B.6.4.1.3-06) cannot be overturned with a reliable *in vivo* cytogenetic study as the provided *in vivo* chromosome aberration study (Shirasu *et al*, 1978a, B.6.4.2.1-01) is deemed as supporting information only (abstract from a publication). Results from two *in vivo* Comet assays (Brendler-Schwaab, 2000, B.6.4.2.2-01; Sasaki *et al*, 1997, B.6.4.2.2-02) are contradictory and both studies contain methodology deficiencies, hence deemed as supporting information. Based on the weight of evidence from additional *in vivo* studies in germ cells, negative results were obtained in two dominant lethal tests (Kaneda *et al*, 1978, B.6.4.3.1-01; Shirasu *et al*, 1978, B.6.4.3.1-02) although based on method deficiencies they are deemed as supporting information.

In conclusion, the weight of evidence suggests *ortho*-phenylphenol mutagenicity in germ cells *in vivo* cannot be addressed due to the high uncertainty of the available studies and the potential aneugenicity of *ortho*-phenylphenol has not been suitably addressed with a reliable *in vivo* cytogenetic study. **Therefore, an overall assessment for the genotoxicity of *ortho*-phenylphenol cannot be derived.**

***Ortho*-Phenylphenol sodium salt (SOPP):**

New studies have been included for the renewal assessment of SOPP. A total of 7 studies have been submitted for the evaluation of the genotoxicity of *ortho*-phenylphenol sodium salt, of which a total of 3 correspond to studies *in vitro* and 4 correspond to studies *in vivo*. One new study *in vitro* (Ames test) and three new studies *in vivo* (chromosome aberration, Comet and UDS assays) have been provided as part of the renewal evaluation processed, thus two studies were presented and evaluated as part of the original DAR (2008).

No photomutagenicity study was provided. Whilst photomutagenicity testing is potentially triggered, the *in vitro* 3T3 NRU phototoxicity assay performed with OPP returned a negative result (Leuschner, 2018, B.6.2.7). In the buffered cell culture test (Balb/3T3 cells) OPP and SOPP are expected to be equivalent and present the same chromophore and absorption UV/VIS spectrum, thus no photomutagenicity testing is considered to be necessary.

***In vitro*:**

SOPP showed negative results in *S.typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 (San and Springfied, 1989b, B.6.4.1.1-10). This study did not include a strain to test for cross-linking mutagens. No further studies on bacterial mutagenicity were submitted for SOPP.

Negative results were reported for SOPP in *in vitro* chromosome aberration tests in CHO-k1 cells in the absence of metabolic activation (Ishidate *et al*, 1983, B.6.4.1.3-03; Ishidate *et al*, 1988, B.6.4.1.3-04). These publications only contained result data or the study did not use metabolic activation, hence, they are considered as supporting information only.

***In vivo*:**

SOPP was negative in a cytogenetic study in murine bone marrow cells of rats and mice (Yoshida *et al*, 1979, B.6.4.2.1-02). Based on methodology deficiencies, this study is only deemed as supporting information.

SOPP did not induce DNA strand breaks in hepatocytes or stomach cells in an *in vivo* Comet assay (De Boeck *et al*, 2015, B.6.4.2.2-03). Despite a few deviations from current OECD TG 489 (2016), which include purity of the test substance, the number of total cells per organ was less than 150 and body weights not recorded at the start and at the end of the study, the RMS deems the study acceptable. No GLP compliance was reported either.

Repeated oral exposure to SOPP in the diet during 3-5 months showed DNA damage in the bladder epithelial cells in a DNA alkaline elution assay *in vivo* (Morimoto *et al*, 1989, B.6.4.2.3-02).

SOPP induced UDS *in vivo* in urinary bladder epithelial cells (Klein, 1986, B.6.4.2.3-04). The RMS deems the

study as supporting information due to the deviations from the OECD TG 486 (1997).

ortho-Phenylphenol classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). No classification for germ cell mutagenicity is included.

Based on all the available genotoxicity data, both bacterial gene mutation and clastogenicity *in vitro* has not been adequately addressed. The Ames test provided (San and Springfied, 1989b, B.6.4.1.1-10) did not assess cross-linking mutagens. As for clastogenicity, the *in vitro* chromosome aberration test did not use metabolic activation (Ishidate *et al*, 1983, B.6.4.1.3-03), hence the assay is incomplete. SOPP is reported negative in a cytogenetic study in rats and mice (Yoshida *et al*, 1979, B.6.4.2.1-02) although the study is deemed as supporting information based on method deficiencies. SOPP is negative in an *in vivo* Comet assay (De Boeck *et al*, 2015, B.6.4.2.2-03) whereas it induced UDS *in vivo* in urinary bladder epithelial cells (Klein, 1986, B.6.4.2.3-04). **In conclusion, based on the available information an overall assessment for the genotoxicity of *ortho*-phenylphenol sodium salt cannot be derived.**

2.6.4.2 Comparison with the CLP criteria regarding genotoxicity / germ cell mutagenicity

No human data are available for *ortho*-phenylphenol (OPP), hence a classification as Category 1 is not possible.

The classification in Category 2 is based on:
- positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from:
- somatic cell mutagenicity tests *in vivo*, in mammals; or
- other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays.

All available *in vivo* germ and somatic cells mutagenicity assay data do not meet the criteria for classification. However, based on the low reliability of these data and on the undetermined evaluation of the clastogenicity *in vivo*, the conclusion for no classification and labelling cannot be drawn (**data gap**).

2.6.4.3 Conclusion on classification and labelling for genotoxicity / germ cell mutagenicity

Based on the data available for ***ortho*-phenylphenol (OPP)** and according to the criteria under Regulation (EC) No 1272/2008, **no conclusion for the classification of genotoxicity / germ cell mutagenicity can be drawn (data inconclusive).**

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)	**Negative (± S9)**	Pagano *et al*., 1988

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		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 No information on test concentrations and no result table available Supporting information	**Negative (± S9)**	Shirasu *et al.,* 1978a B.6.4.1.1-03
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 OPP purity > 99% L5178Y TK +/- Solvent: water (-S9), DMSO (+S9) 20-60 µg/mL (-S9) 0.32-5 µg/mL (+S9) Study acceptable	**Negative (+S9)**	NTP, 1986 B.6.4.1.2-04
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	**Negative (-S9)**	Ishidate *et al.*, 1984 B.6.4.1.3-01

OPP: purity not stated Chinese hamster lung fibroblasts (CHL) Solvent: DMSO Up to 0.05 mg/mL 48h expression time Supporting information		
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 Solvent: DMSO Chinese hamster lung fibroblasts (CHL) 100 µg/mL, 3h treatment Compilation of experimental results from publications OPP	**Positive (-S9) CHO-K1 cells** **Negative (-S9) CHL cells**	Ishidate *et al.,* 1988 B.6.4.1.3-04
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 induced SCE's and chromosome aberrations (+S9)** **PHQ induced chromosome aberrations**	Tayama *et al.,* 1989 B.6.4.1.3-05

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	**(+S9) and SCE's (± S9)**	
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 Purities not stated Chinese hamster V79 lung fibroblasts OPP: 50-400 µM PHQ: 25-45 µM PBQ: 20-30 µM Supporting information	**OPP itself did not cause DNA single strand breaks** or 8-OHdG formation. **PHQ and PBQ caused a significant increase in both parameters** at non-cytotoxic concentrations	Henschke *et al.,* 2000 B.6.4.1.4-01

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
DNA reactivity by formation of 8-OHdG No guidance GLP: no Supporting information ortho-Phenylphenol Phenylbenzoquinone Phenylhydroquinone	**PHQ caused a dose dependent increase in 8-OHdG** EDTA (oxygen radical scavenger) inhibits the	Nagai *et al.,* 1995 B.6.4.1.4-07

Purity not stated Calf thymus DNA Concentrations: 10-5 to 10-2 M CuCl2 and FeCl2 concentrations: 5 µM	PHQ-induced formation of 8-OHdG. $CuCl_2$ had an effect in PHQ-dependent DNA cleavage	
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 Experiment II: 1.0, 3.16, 10.0, 31.6, 100, 316, 1000 and 2500 µg/plate	**Negative**	STUGC21AA1248-2 Study submitted by applicant during the consultation
In vitro Mammalian Micronucleus Assay OECD TG 487 GLP: yes	**Negative**	STUGC21AA1248-3 Study submitted by applicant

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)	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	**Negative** in any tested doses (single or repeat exposure)	Shirasu *et al.,* 1978a B.6.4.2.1-01

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		
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. GLP: no Purity not stated Male CD-1 mice Liver, lung, kidney, spleen, brain, bladder and bone marrow Volume: 10 mL olive oil	**OPP induced DNA damage in the stomach, liver, kidney, bladder and lung**	Sasaki *et al.,* 1997 B.6.4.2.2-02

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		
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% Volume: 0.4 mL Male F344/DuCrj rats Urinary bladder epithelium Vehicle: 0.9% NaCl solution Intravesical injection into the bladder	**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

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
Dominant Lethal test Pre-guidance Deviations from current OECD TG 478: only abstract provided; no adequate study description. GLP: no	**Negative**	Shirasu *et al.,* 1978a B.6.4.3.1-02

OPP: purity not stated C3H male mice 0, 100 and 500 mg/kg bw Oral Duration of exposure: 5 days Supporting information		
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.**

2.6.5 Summary of long-term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template]

Table 53: Summary table of animal studies on long-term toxicity and carcinogenicity.

Method. Guideline, deviations if any. Acceptability. Strain/Species. No of animals.	**Test substance Dose levels, duration of**	**Results:** **- LOAEL** **- NOAEL** **- Critical effects at the LOAEL** **- Target tissue/organ** **[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)]**	**Reference**
Long-term study. No guideline. **Supportive**	OPP (purity 98%) Dietary	*Mortality:* no evidence of any treatment related effect (ranged from 68 to 88%, ndr., data not shown) **20000 ppm** (≈1000-2000 mg/kg bw/day)	Hodge *et al.* (1952) (CA) B.6.5/01

Method. Guideline, deviations if any. Acceptability. Strain/Species. No of animals.	**Test substance Dose levels, duration of**	**Results:** **- LOAEL** **- NOAEL** **- Critical effects at the LOAEL** **- Target tissue/organ** **[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)]**	**Reference**
only. Wistar-derived rat. Both sexes. 25/sex and dose.	0, 200, 2000, 20,000 ppm, for 2-years.	Bodyweight: ▪ ↓ bw in ♂/♀ (10%/6%) in moth 12. Organ weights: ▪ Testes: ↑ abs wt at sacrifice (46%). Histopathology: *Non-neoplastic changes* Kidney ▪ Extensive renal damage, characterised by tubular dilatation with varying degrees of acute and chronic inflammation (data not shown). **2000 ppm** (≈100-200 mg/kg bw/day) Organ weights: ▪ Testes: ↑ abs wt at sacrifice (17%, ns). **200 ppm** (≈10-20 mg/kg bw/day) Organ weights: ▪ Testes: ↑ abs wt at sacrifice (25%, ns). -**LOAEL**=20,000 ppm (≈1000-2000 mg/kg bw/day) -**NOAEL**=2000 ppm (≈100-200 mg/kg bw/day) -Critical effect at the LOAEL: renal damage (♂,♀), ↓ body weights (♂,♀), ↑ testes weights. **Target tissue/organ: Kidneys.**	
Combined Chronic Toxicity/carcinogenicity. OECD Guideline 453. Deviations: Age at study start older than recommended. No satellite groups. Water consumption not measured. Volume of urine not recorded. **Accepted**. Fischer 344rats. Both sexes. 20/sex and dose in the 1-year group. 50/sex and dose in the 2-year group.	OPP, (purity 99.7-100%) Dietary 0, 800, 4000, 8000/10,000 ppm for ♂/♀ (39/49, 200/248 and 402/647 mg/kg bw/day for ♂/♀) for up to 2-years.	*Mortality:* no evidence of any treatment related effect. **8000/10,000 ppm** ♂/♀ (402/647 mg/kg bw/day) Bodyweight: ▪ ↓ bw in ♂/♀ [week 5 (14%/8.4%), week 10 (10%/8%), week 20 (9%/8%), week 30 (11%/9%), week 40 (9%/9%), week 50 (10%/10%), week 60 (10%/11%), week 70 (11%/14%), week 80 (12%/15%), week 90 (15%/16%), week 100 (13%/15%) and week 104 (10%/15%)] ▪ ↓ bw gain in ♂/♀ [week 5 (29%/31%), week 30 (6%/-), week 70 (132%, ndr/143%) and week 90 (147%/-, ndr)] Clinical signs: ▪ Abnormal colour urination in ♂ and urine stains (red and brown) in ♂ and ♀. Ophthalmology: ▪ ↑ incidence of cataracts in ♂ (60%) Haematology: ▪ ↑ MCV in ♂ [3-months (2%)], ↓ in ♀ [6-month (1%, ndr)] ▪ ↑ MCH ♂ [3-months (2%), 6-months (3%)] and ↓ in ♀ [12-month (2%)] ▪ ↑ MCHC ♂ [6-months (2%)] ▪ ↓ RBC ♂ [12-months (2%, ndr)] ▪ ↓ Hct ♀ [12-months (3%, ndr)] ▪ ↓ Hgb ♀ [12-months (3%, ndr)] Clinical chemistry: ▪ ↑ Cl ♂ (3%) ▪ ↑ BUN ♀ (27%) ▪ ↓ Uric-A ♂/♀ (33%/33%) ▪ ↓ Trig ♂/♀ (61%/56%) ▪ ↓ Chol ♂ (51%) ▪ ↑ ALP ♂ (36%) ▪ ↓ T-Bili ♂/♀ (67%/67%)	Wahle & Christenson (1996) (CA) B.6.5-02

Method. Guideline, deviations if any. Acceptability. Strain/Species. No of animals.	Test substance Dose levels, duration of	**Results:** **- LOAEL** **- NOAEL** **- Critical effects at the LOAEL** **- Target tissue/organ** **[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)]**	Reference
		▪ ↑ Alb ♂ (10%) ▪ ↓ Glob ♂ (11%) <u>Urinalysis:</u> ▪ ↓ Protein in ♂/♀ [3-months (40%/56%), 6-month (71%/61%), 12-month (77%/74%), 18-month (70%/90%) and 24-month (75%/86%)] ▪ ↑ pH ♂/♀ [6-months (-/8%), 12-months (-/5%) and 18-months (3%/4%,ncdr)] ▪ ↓ Ketones in ♂/♀ [3-months (55%/-), 6-month (38% /67%), 12-month (20%/-), and 24-month (-/100%)] ▪ ↑ blood in ♂ [18-months (700%) and 24 months (∞ %) ▪ ↓ specific gravity in ♂/♀ [3-months (1%/-), 6-months (2%/1%), 12-months (1%/-) and 18-months (-/1%)] ▪ ↓ leukocytes in ♂/♀ (3-months (100%/-) , 6-month (100% /-), 12-month (67%/0%), 18-month (50%/100%) and 24-month (50%/68%). <u>Organ weights:</u> ▪ Heart : ↓ abs. wt. in ♂/♀ [1-year (10%/-) and 2-years (9%/7%)] and ↑ rel. wt. in ♀ [2-years (9%)] ▪ Testes: ↑ abs. wt. [2-years (34%)] and ↑ rel. wt. [1-year(12%) and 2-years(46%)] ▪ Adrenals : ↓ abs. wt. in ♀ [1-year (8%, ndr) and 2-years (14%)] ▪ Kidney : ↓ abs. wt. in ♀ [2-years (11%)] ▪ Liver :↓ abs. wt. in ♀ [2-years (13%)] and ↑ rel. wt. in ♂/♀ [1-year (7%/-) and 2-years (14%, ndr)] ▪ Brain : ↑ rel. wt. in ♂/♀ [1-year (9%/8%) and 2-years (8%/18%)] ▪ Lungs: ↑ rel. wt. in ♂/♀ [1-year (5%, ndr/-) and 2-years (-/10%)] <u>Gross pathology:</u> ▪ ↑ incidence of urinary bladder masses in ♂ (74% vs. 0% in controls) ▪ ↑ incidence of pitted zones in kidneys in ♀ (14% vs. 0% in controls) ▪ ↑ incidence of abnormal texture in kidneys in ♀ (16% vs. 2% in controls) ▪ ↑ incidence of wet/stained ventrum in ♂/♀ (44% vs. 3% in controls/44% vs. 8% in controls) <u>Histopathology:</u> ▪ ↑ incidence urinary bladder pathologies: ○ ↑ incidence of transitional cell carcinomas in ♂ at 24 months (34/50 vs. 0/50) ○ ↑ incidence of papillomas in ♂ at 12 months (6/20 vs. 0/20) and at 24 months (6/50 vs. 0/50) ○ ↑ incidence of nodular/papillary hyperplasia in ♂ at 12 months (20/20 vs. 0/20) and 24 months (43/50 vs. 1/50). ○ ↑ incidence of simple hyperplasia in ♂ at 12 months (20/20 vs. 0/20) and in ♂/♀ at 24 months (42/50 vs. 2/50 /6/50 vs. 0/50). ○ ↑ incidence of calculus in ♂ at 12 months (16/20 vs 8/20 in controls), and at 24 months (21/50 vs. 3/50 in controls). ○ ↑ incidence of congestion in ♂at 24 moths (16/50 vs. 1/50). ○ ↑ incidence of haemorrhage in ♂ at 24 moths (9/50 vs. 0/50). ○ ↑ incidence of mineralization in ♂ at 24 moths (18/50 vs. 3/50). ○ ↑ incidence of necrosis in ♂ at 24 moths (20/50 vs. 3/50). ○ ↑ cyst in ♀ at 12 months (5/20 vs 0/20 in controls). ▪ ↑ incidence kidney pathologies: ○ ↑ Incidence calculus in ♀ at 24 months (21/50 vs. 16/50, ns.; ndr) ○ ↑ Incidence of 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) ○ ↑ Incidence infarct in ♀ at 24 months (29/50 vs. 3/50)	

Method. Guideline, deviations if any. Acceptability. Strain/Species. No of animals.	Test substance Dose levels, duration of	**Results:** **- LOAEL** **- NOAEL** **- Critical effects at the LOAEL** **- Target tissue/organ** **[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)]**	Reference
		○ ↑ Incidence acute inflammation in ♀ at 24 months (11/50 vs. 2/50) ○ ↑ Incidence papilla mineralisation in ♀ at 24 months (12/50 vs. 0/50) **4000 ppm** (200/248 mg/kg bw/day ♂/♀) <u>Bodyweight:</u> ▪ (↓) bw in ♂/♀ [week 5 (6%/4%), week 10 (4%/4%), week 20 (4%/5%), week 30 (5%/5%), week 40 (4%/5%), week 50 (5%/4%), week 60 (5%/3%), week 70 (5%/6%), week 80 (6%/5%), week 90 (6%/5%) and week 100 (4%/- %)] ▪ (↓) bw gain in ♂/♀ [week 5 (13%/14%), week 10 (8%/-, ndr), week 20 (-/22%, ndr), week 30 (39%/-), week 70 (-/98%) and week 90 (102%/-, ndr)] and (↑) bw gain in ♂ in week 104 (106%, ndr). <u>Clinical signs:</u> ▪ Urine stains in ♀. <u>Ophthalmology:</u> ▪ ↑ incidence of uveitis (21%, ndr), corneal neovascularization (21%, ndr) and cataracts (27%, ndr) in ♀. <u>Haematology:</u> ▪ ↑ MCV in ♂ [3-months (2%)], ↓ in ♂/♀ [6-months (-/2%, ndr), 18-months (-/1%)] ▪ ↑ MCH ♂ [3 months (1%), 6-months (2%)] and ↓ in ♀ [12-month (2%), 18-months (2%)] ▪ ↑ MCHC ♂ [6-months (1%)] ▪ ↓ PLT ♂ [18-months (12%, ndr) <u>Clinical chemistry:</u> ▪ ↑ Cl ♂ (1%) ▪ ↓ Uric-A ♂/♀ (17%/17%) ▪ ↓ Trig ♂ (45%) ▪ ↓ Chol ♂ (36%) ▪ ↓ T-Bili ♂ (33%) ▪ ↑ Alb ♂ (7%) <u>Urinalysis:</u> ▪ ↓ Protein ♂/♀ [3-months (-/48%), 6-month (64%/64%), 12-month (56%/69%), 18-month (42%/79%) and 24-month (23%/50%)] ▪ ↓ Ketones in ♂/♀ [3-months (55%/-) and 6-month (38% /67%)] ▪ ↓ specific gravity in ♂/♀ at 6-months (1%/1%) ▪ ↓ leukocytes in ♂/♀ (3-months (100%/-) , 6-month (100%/-), 12-month (33%/0%), 18-month (25%/50%) and 24-month (25%/33%) <u>Organ weights:</u> ▪ ↓ adrenals abs. wt. in ♀ [1-year (11%, ndr)] ▪ ↓ kidney abs. wt. in ♀ [2-years (8%)] ▪ ↓ liver abs. wt. in ♀ [2-years (10%)] ▪ ↑ testes rel. wt. [1-years (9%)] <u>Gross pathology:</u> ▪ ↑ Incidence of urinary bladder masses in ♂ (4% vs. 0% in controls; n.s.). <u>Histopathology</u>: *Non-neoplastic changes:* ▪ ↑ incidence kidney pathologies: ○ ↑ incidence of calculus in kidney♀ at 24 months (33/50 vs. 16/50, ndr) ▪ ↑ Incidence of simple hyperplasia in urinary bladder ♂ at 24 months (6/50 vs. 2/50 in control; n.s.).	

Method. Guideline, deviations if any. Acceptability. Strain/Species. No of animals.	Test substance Dose levels, duration of	Results: - LOAEL - NOAEL - Critical effects at the LOAEL - Target tissue/organ [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)]	Reference
		Neoplastic changes: *Urinary bladder* ▪ ↑ Incidence of transitional cell carcinomas in ♂ at 24 months (2/50 vs. 0/50 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.). **800 ppm** (39/49 mg/kg bw/day ♂/♀) Bodyweight: ▪ ↓ bw in ♀ [week 10 (3%), week 20 (3%) and week 30 (3%)]. ▪ ↓ bw gain in ♀ [week 5 (7%) and week 70 (55%, ndr)] and in ♂ [week 40 (72%, ndr) and week 90 (108%, ndr)]. Clinical signs: ▪ Urine stains in ♀. Haematology: ▪ ↑ MCH ♂/♀ [3-months (1%/-), 12-month (-/1%, ndr)] ▪ ↓ PTL ♀ [12-months (6%, ndr) Histopathology: ▪ ↑ Incidence of calculus in kidney♀ at 24 months (27/50 vs. 16/50, ndr) Urinalysis: ▪ ↓ Ketones in ♂ [3-months (36%) and 6-month (38%)] ▪ ↓ Leukocytes in ♂ [3-months (100%), 6-month (50%) and 18-month (0%)] -**Systemic LOAEL**= 4000 ppm (200 mg/kg bw/day) -**Systemic NOAEL**= 800 ppm (39 mg/kg bw/day) -Critical effect at the LOAEL: structural alterations in the urinary bladder (♂). -**Neoplastic LOAEL**= 8000 ppm (402 mg/kg bw/day) -**Neoplastic NOAEL**= 4000 ppm (200 mg/kg bw/day) -Critical effect at the LOAEL: neoplasms (malignant and benign) in the urinary bladder (♂) -**Target tissue/organ:** Urinary bladder	
Long-term study OECD Guideline 453. Deviations: No satellite groups. Incomplete testing and reporting. **Supportive only.** F344/DuCrj rats. Males. 20-24/ Dose group.	OPP (purity 98%) Dietary 0, 6250, 12,500, 25,000 ppm (269, 531 and 1140 mg/kg bw/day), for 91-weeks.	*Mortality*. Mortality rates in 12500 and 25000 ppm OPP treated groups were significantly higher than that of controls, 29% and 35% higher respectively. **25,000 ppm** (≈1140 mg/kg bw/day) Bodyweight: ▪ ↓ bw [from week 21 to the end of the study (17-24%, numerical data unavailable)]. Food intake: ▪ ↓ Abs. food intake (from weeks 1 to 85, except at week 33) and ↑ rel. food intake (throughout the study, except form weeks 3 to 13). Clinical observations ▪ Occult blood in the urine (from week 15 onwards) ▪ Gross haematuria (from week 52 onwards) Hyperplastic or neoplastic lesions of the urinary bladder: **Hyperplastic or neoplastic lesions of the urinary bladder**	Hiraga & Fujii (1984) (CA) B.6.5-03

<table>
<tr><th>Method. Guideline, deviations if any. Acceptability. Strain/Species. No of animals.</th><th>Test substance Dose levels, duration of</th><th>Results:
- LOAEL
- NOAEL
- Critical effects at the LOAEL
- Target tissue/organ

[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)]</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><th rowspan="2">OPP [ppm]</th><th rowspan="2">Rats examined</th><th rowspan="2">Rats with bladder tumour</th><th colspan="3">No. of rats with transitional cell …</th></tr>
<tr><th>Hyperplasia</th><th>Papilloma</th><th>Carcinoma
Non-invasive</th><th>Carcinoma
Invasive</th></tr>
<tr><td>0</td><td>24</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>6250</td><td>20</td><td>0</td><td>2</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>12,500</td><td>24</td><td>23*</td><td>0</td><td>3</td><td>15</td><td>5</td></tr>
<tr><td>25,000</td><td>23</td><td>4</td><td>7</td><td>2</td><td>2</td><td>0</td></tr>
</table>
* Significantly different from controls, p <0.001

12,500 ppm (531 mg/kg bw/day)

<u>Bodyweight:</u>
▪ ↓ bw [from week 21 to the end of the study (2-12%, numerical data unavailable)].
<u>Clinical observations</u>
▪ Gross haematuria (from week 52 onwards)
<u>Hyperplastic or neoplastic lesions of the urinary bladder:</u>
▪ ↑ of bladder tumours (23/24 vs. 0/24 in controls).

-Systemic LOAEL=12,500 ppm (531 mg/kg bw/day)
-Systemic NOAEL=6250 ppm (269 mg/kg bw/day)
-Critical effect at the LOAEL: ↑ mortality and ↓ bw.

-Neoplastic LOAEL=12,500 ppm (531 mg/kg bw/day)
-Neoplastic NOAEL=6250 ppm (269 mg/kg bw/day)
-Critical effect at the LOAEL: transitional cell papilloma and carcinoma in the urinary bladder.

Target tissue/organ: Urinary bladder.
</td><td></td></tr>
<tr><td>Dietary in, mouse.
OECD Guideline 453.
Deviations: No satellite groups. Haematology, clinical biochemistry and urinalyses determinations were only performed on terminal samples instead of at 3 and 6 months. More haematological parameters should have been measured. No</td><td>OPP (purity 99.88%)
Dietary 0, 250, 500, 1000 mg/kg bw/day, for 2-years.</td><td>
Mortality: not affected by OPP treatment.

1000 mg/kg bw/day
<u>Bodyweight:</u>
▪ ↓ bw in ♂/♀ [day 63 (-/3%), day 147 (3%/5%), day 343 (12%/16%), day 511 (14%/21%), day 707 (14%/15%) and day 728 (13%/20%)].
▪ ↓ bw gain in ♂/♀ [day 63 (-/17%), day 147 (11%/16%), day 343 (26%/32%), day 511 (28%/37%), day 707 (27%/41%) and day 728 (28%/38%)].
<u>Urinalysis:</u>
▪ ↓ specific gravity in ♀ [24-months (2.4%)]
<u>Organ weights at 24 months:</u>
▪ Heart : ↓ abs. wt. in ♂ (13%) and ↑ rel. wt. in ♀ (14%)
▪ Kidney ↑ rel. wt. in ♀ (20%)
▪ Brain : ↑ abs. wt. in ♂ (2%) and ↑ rel. wt. in ♀ (23%).
<u>Organ weights at 12 months:</u>
▪ Heart: ↓ abs. wt. in ♂ (14%) and ↑ rel. wt. in ♀ (28%)
▪ Adrenals : ↑ rel. wt. in ♀ (15%).
▪ Kidney : ↓ abs. wt. in ♂ (18%) and ↑ rel. wt. in ♀ (29%)
▪ Liver: ↑ rel. wt. in ♀ (61%) and ↑ abs. wt. in ♂/♀ (25%/27%)
▪ Brain: ↑ rel. wt. in ♂/♀ (14%/26%)
▪ Testes: ↑ rel. wt. (14%)
<u>Histopathology:</u>
*Statistically identified difference from control mean by Yate´s Chi-Square pairwise test, alpha=0.10, two-sided; alpha=0.05, one-sided.
</td><td>Quast & McGuirk (1995) (CA) B.6.5-04</td></tr>
</table>

Method. Guideline, deviations if any. Acceptability. Strain/Species. No of animals.	Test substance Dose levels, duration of	Results: - LOAEL - NOAEL - Critical effects at the LOAEL - Target tissue/organ [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)]	Reference

statistical analysis on gross pathology data.
Accepted.
B6C3F1 mice.
Both sexes.
60/sex and dose.

(T) Linear trend by Cochran-Armitage linear trend test, alpha=0.02, two-sided; alpha=0.01, one sided.

Organ and Lesion (Lesion rate in %)	Dose (mg/kg bw/day) Male 0	250	500	1000	Female 0	250	500	1000
Bone (No examined)	50	10	13	50	48	20	22	50
Fibrous osteodystrophy	0 (0%)	0 (0%)	0 (0%)	0 (0%)	8 (17%)	12 (60%)	9 (41%)	21* (42%)
Kidneys (No. examined)	50	50	50	50	48	50	50	50
Mineralization tubule (very slight)	49 (98%)	47 (94%)	46 (92%)	39*T (78%)	31 (65%)	27 (54%)	21 (42%)	28 (56%)
Degeneration/regeneration tubule (very slight)	17 (34%)	35* (70%)	34* (68%)	38*T (76%)	27 (56%)	16 (32%)	20 (40%)	20 (40%)
Degeneration/regeneration tubule (slight)	26 (52%)	9* (18%)	3* (6%)	3*T (6%)	2 (4%)	0 (0%)	2 (4%)	1 (2%)
Vacuolation-decreased tubule (very slight) (Lesion rate)	5 (10%)	11 (22%)	0* (0%)	0* (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Vacuolation-decreased tubule (slight)	3 (6%)	25* (50%)	5 (10%)	0* (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Vacuolation-decreased tubule (moderate)	1 (2%)	5 (10%)	31* (62%)	21* (42%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Vacuolation-decreased tubule (severe)	6 (12%)	9 (18%)	14* (28)	29*T (58%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Vacuolation-decreased tubule (any severity)	15 (30%)	50* (100%	50* (100%	50* (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Liver (No examined)	50	50	50	50	48	50	50	50
Accentuated lobular pattern (very slight)	8 (16%)	17* (34%)	4 (8%)	0* (0%)	3 (6%)	12* (24%)	15* (30%)	4 (8%)
Accentuated lobular pattern (slight)	3 (6%)	16* (32%)	20* (40%)	11* (22%)	2 (4%)	2 (4%)	10* (20%)	19*T (38%)
Accentuated lobular pattern (moderate)	1 (2%)	1 (2%)	11* (22%)	26*T (52%)	2 (4%)	0 (0%)	1 (2%)	14* (28%)
Accentuated lobular pattern (any severity)	12 (24%)	34* (68%)	35* (70%)	37* (74%)	7 (15%)	14 (28%)	26* (52%)	37*T (74%)
Focus of altered cells-eosinophilic, hepatocel., multifocal	1 (2%)	1 (2%)	5 (10%)	9*T (18%)	1 (2%)	0 (0%)	1 (2%)	2 (4%)
Focus of altered cells-eosinophilic, hepatocel., focal or multifocal	3 (6%)	6 (12%)	12* (24%)	16*T (32%)	2 (4%)	1 (2%)	5 (10%)	6 (12%)
Focus of altered cells-vacuolated o clear, multifocal	4 (8%)	6 (12%)	1 (2%)	0T (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Focus of altered cells-vacuolated o clear, focal or multifocal	7 (14%)	10 (20%)	3 (6%)	0*T (0%)	5 (10%)	2 (4%)	0* (0%)	0*T (0%)
Vacuolation with fatty change (slight)	5 (10%)	7 (14%)	3* (6%)	0*T (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Vacuolation with fatty change (any severity)	10 (20%)	7 (14%)	3* (6%)	0*T (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Necrosis hepatocellular (any severity)	5 (10%)	4 (8%)	1 (2%)	1 (2%)	8 (17%)	6 (12%)	2* (4%)	2*T (4%)

500 mg/kg bw/day

Bodyweight:

- ↓ bw in ♂/♀ [day 63 (-/3%), day 343 (6%/7%), day 511 (5%/10%), day 707 (7%/16%) and day 728 (-/13%)].
- ↓ bw gain in ♂/♀ [day 63 (-/12%), day 343 (13%/13%), day 511 (10%/18%), day 707 (17%/30%) and day 728 (-/25%)] and ↑ bw gain in ♂ [day 63 (12%, ndr)]

Urinalysis:

- ↓ specific gravity in ♀ [24-months (2%)]

Organ weights (24-months):

- Heart : ↓ abs. wt. in ♂ (8%) and ↑ rel. wt. in ♀ (15%)
- Kidney : ↑ rel. wt. in ♀ (18%)

<table>
<tr><th>Method. Guideline, deviations if any. Acceptability. Strain/Species. No of animals.</th><th>Test substance Dose levels, duration of</th><th>Results:
- LOAEL
- NOAEL
- Critical effects at the LOAEL
- Target tissue/organ

[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)]</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
▪ Brain : ↑ rel. wt. in ♀ (15%)
<u>Organ weights at 12 moths:</u>
▪ Heart : ↓ abs. wt. in ♂ (11%).
▪ Kidney : ↓ abs. wt. in ♂ (14%) and ↑ rel. wt. in ♀ (14%)
▪ Liver : ↑ abs/rel. wt. in ♀ (17%/28%))
▪ Brain : ↑ rel. wt. in ♂ (15%)
▪ Testes: ↑ rel. wt. (13%)
<u>Tumour incidence:</u>
<table>
<tr><th></th><th colspan="4">Males</th><th colspan="4">Females</th></tr>
<tr><th></th><th>0</th><th>250</th><th>500</th><th>1000</th><th>0</th><th>250</th><th>500</th><th>1000</th></tr>
<tr><td>Number of mice examined</td><td>50</td><td>50</td><td>50</td><td>50</td><td>48</td><td>50</td><td>50</td><td>50</td></tr>
<tr><td>Type of tumour</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>1) Adenoma, hepatocellular</td><td>27</td><td>33</td><td>40*</td><td>41*</td><td>13</td><td>14</td><td>17</td><td>19</td></tr>
<tr><td>2) Carcinoma, hepatocellular</td><td>11</td><td>5</td><td>14</td><td>12</td><td>2</td><td>8</td><td>6</td><td>5</td></tr>
<tr><td>3)Hepatoblastoma, malignant</td><td>0</td><td>2</td><td>6</td><td>3</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>2) + 3) combined</td><td>11</td><td>7</td><td>19</td><td>15</td><td>2</td><td>8</td><td>6</td><td>5</td></tr>
<tr><td>1) + 2) + 3) combined</td><td>32</td><td>36</td><td>45*</td><td>43*</td><td>15</td><td>22</td><td>23</td><td>24</td></tr>
</table>
* Statistically different from control mean by χ^2 pairwise test, α=0.10, two-sided, α=0.05, one-sided

250 mg/kg bw/day
<u>Bodyweight:</u>
▪ ↓ bw in ♀ [day 63 (3%), day 511 (6%) and day 707 (9%)].
▪ ↓ bw in ♀ [day 511 (10%) and day 707 (16%)]
<u>Organ weights at 12 moths:</u>
▪ Kidney: ↑ rel. wt. in ♀ (13%)
▪ Liver: ↑ abs/rel. wt. in ♀ (9%/12%))

-Systemic LOAEL = 250 mg/kg bw/day.
-Systemic NOAEL < 250 mg/kg bw/day
-Critical effect at the LOAEL: ↑ liver weights, changes in hepatocytes and tubule morphology (♂,♀), ↓ bw/bwg (♀).

-Neoplastic LOAEL= 500 mg/kg bw/day
-Neoplastic NOAEL= 250 mg/kg bw/day
-Critical effect at the LOAEL: ↑ incidence of hepatocellular adenoma (♂).

Target tissue/organ: Liver and kidney to a lesser extent.
</td><td></td></tr>
<tr><td>Long-term dermal, mouse.
No guideline.
Supportive only.
Swiss CD-1 mice
Both sexes.
50/sex and dose.</td><td>OPP purity >99%)
0, 55.5 mg/animal / day, 3 days a week, (with or without 0.05 mg of DMBA pre-treatment) for 102 weeks.
An additional positive</td><td>
Mortality: There were no significant group differences in survival attributable to OPP treatment. None of the animals survived until scheduled sacrifice at week 104.

Pathology:
▪ Skin: Non-neoplastic lesions in ♂ and ♀ (ulcers, active chronic inflammation, hyperkeratosis, and acanthosis) at the site of application in all groups, with an increased incidence in male and female mice of the OPP, DMBA/OPP, or DMBA/TPA treatment groups (see table below).
▪ ↑ incidence of basal cell tumours or basal cell carcinomas in ♂ in the DMBA/OPP group (considered to be related to DMBA administration rather than OPP)

Incidence of skin lesions at the application site
<table>
<tr><th rowspan="2">Lesion</th><th colspan="2">Acetone</th><th colspan="2">OPP</th><th colspan="2">DMBA</th><th colspan="2">DMBA/ OPP</th><th colspan="2">DMBA/ TPA</th></tr>
<tr><th>M</th><th>F</th><th>M</th><th>F</th><th>M</th><th>F</th><th>M</th><th>F</th><th>M</th><th>F</th></tr>
<tr><td>Ulcer</td><td>5</td><td>1</td><td>19</td><td>11</td><td>2</td><td>7</td><td>16</td><td>11</td><td>15</td><td>12</td></tr>
</table>
</td><td>Toxicology Program, (1986) (CA) B.6.5/05</td></tr>
</table>

Method. Guideline, deviations if any. Acceptability. Strain/Species. No of animals.	Test substance Dose levels, duration of	Results: - LOAEL - NOAEL - Critical effects at the LOAEL - Target tissue/organ [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)]	Reference
	control group was treated with: 0.05 mg DMBA, then 0.005 mg of TPA Range finding: see B.6.3.3/03		

Active chronic inflammation	10	7	25	20	10	7	25	27	27	25
Hyperkeratosis	7	4	27	16	8	4	24	27	30	26
Acanthosis	13	4	44	36	12	12	33	42	44	41
Squamous cell papilloma	0	0	0	0	1	4	4	2	7	17
Squamous cell carcinoma	0	0	0	0	4	3	1	3	18	18
Basal cell tumour	0	0	0	0	1	0	2	0	1	0
Basal cell carcinoma	0	0	0	0	0	2	2	3	0	2
Keratoacanthoma	0	0	0	0	0	0	0	0	1	5
Sebaceous adenoma	0	0	0	0	1	1	1	0	0	0
Sebaceous adenocarcinoma	0	0	0	0	0	1	0	0	0	0
Neoplastic skin lesion (combined)	0	0	0	0	6	9	9	8	19	32

For statistical analysis of skin lesions at the application site see table B.6.5-05/3 in section B.6.5-05.

-**LOAEL** >55.5 mg/ animal/day
-**NOAEL**=55.5 mg/ animal/day

Table 54: Summary table of human data on long-term toxicity and carcinogenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Dermal absorption study.	$^{3}C/^{14}C$-OPP	Six male volunteers participated in the study. A $^{13}C/^{14}C$-OPP solution was applied over the forearm. Each of the six volunteers received approximately 0.4 mg OPP (~ 6 µg/kg bw) and approximately 41.5 µCi of radioactivity.	OPP is rapidly absorbed via skin and excreted predominantly *via* urine (A mean of 42.70 ± 9.82% of the administered dose was excreted in the urine). The vast majority of absorbed material is excreted within the first 24 h after application.	Selim, S. (1996) (CA) B.6.1.2-01
Metabolism study.	OPP and OPP metabolites	The purpose of the study was to characterise the metabolites of OPP present in urine samples from the dermal absorption study described in section B.6.1.2-01 (Selim, S., 1996).	The majority of an absorbed dose of dermally applied OPP is eliminated in the urine, primarily as polar conjugates of OPP or hydroxylated metabolites. Trace levels of unmetabolized parent compound were only found at early sampling intervals. No free PHQ was found in any of the urine samples. OPP, both free and conjugated, accounted for 73.0 % of the total absorbed dose following dermal exposure for 8 h.	Bartels, M. *et al.* (1997) (CA) B.6.1.2-02

Table 55: Summary table of other studies relevant for long-term toxicity and carcinogenicity

Type of study/data	Test substance	Relevant information about the study	Observations	Reference

Type of study/data	Test substance	Relevant information about the study	Observations	Reference
Two-generation, rat. *See table 57 for more information.*	OPP	40, 140 and 490 mg/kg bw/day (Actual doses:35, 125, 457 mg/kg bw/day) for 2 generations. CD Sprague-Dawley rats . Both sexes. At least 25/ Dose group.	-Parental NOAEL = 35 mg/kg bw/day -Critical effect at the LOAEL (125 mg/kg bw/day): bladder calculi (♂), **urothelial hyperplasia*** (♂,♀) **Increased incidence of transitional bladder epithelium cell hyperplasia was detected in males and females of the first parental generation and in F1 males.*	Eigenberg (1990) (CA) B.6.6.1-01
Two-generation, rat. Hodge *et al.* (1952). B.6.3.2-01 *See table 57 for more information.*	OPP	20, 100, 500 mg/kg bw/day (Actual doses: 18/17, 93/92, 459/457 mg/kg bw/day for ♂/♀) for 2 generations. Albino CD Sprague-Dawley rats. Both sexes. 30/sex/dose.	-Parental NOAEL = 93/92 (♂/♀) mg/kg bw/day -Critical effect at the LOAEL(459/457 (♂/♀) mg/kg bw/day): ↓ bw (♂,♀) and ↑ incidence of **transitional cell hyperplasia (simple and nodular**) (♂). *Additionally Two 500 mg/kg bw/day F1 males had malignant lymphoma involving several tissues and were sacrificed. One 100 mg/kg bw/day F1 female had a nephroblastoma. One P male at the highest dose and one F1 female control had a pituitary adenoma.*	Eigenberg & Lake (1995) (CA) B.6.6.1-02
DNA Damage in urinary bladder epithelium.	OPP, 5-OH and PBQ.	0.4 mL of test substance solutions were injected intravesically through the bladder wall at the following concentrations: Compound / Concentration PBQ: 0.0005 %; 0.005 %; 0.05 %; 0.1 % OPP: 0.05 % 5-OH: 0.05 % F344 rats. Males. 2 / treatment group.	-PBQ but not its precursors OPP or PHQ caused DNA damage in the urinary bladder epithelium.	Morimoto, K., *et al*. (1987) (CA) B.6.4.2.3-01
Unscheduled DNA Synthesis (USD) induction in urinary bladder.	SOPP	SOPP was administered via stomach tube to 16 female rats at 100 mg/kg bw. UDS was assessed in urinary bladder cells. BOR:WISW rats. Females. 16.	SOPP induced UDS in urinary bladder epithelial cells. This is likely to be secondary to cytotoxicity and not reflective of DNA repair.	Klein, W. (1986) (CA) B.6.4.2.3-04
Subchronic study into bladder effects.	OPP	1000, 4000 or 12,500 ppm, in diet ad libitum, for 13 weeks CDF[F-344]/BR rats .Males. 20 /group.	OPP caused morphological alterations of the urinary bladder epithelium in the highest dose group. NOAEL = 4000 ppm (~224 mg/kg bw/day).	Christenson *et al.* (1996a) (CA) B.6.8.2-02
Subchronic ^{32}P-post labelling study.	OPP	1000, 4000 or 12,500 ppm, in diet ad libitum, for 13 weeks. CDF[F-344]/BR rats .Males. 22 /group.	Increase of mitotic activity and hyperplasia of the urothelium at dose levels ≥ 8000 ppm. No DNA adducts. NOAEL = 4000 ppm (~285 mg/kg bw/day).	Christenson *et al*. (1996b) (CA) B.6.8.2-03

Type of study/data	Test substance	Relevant information about the study	Observations	Reference
32-week, dietary.	OPP SOPP	12,500 ppm (OPP) 20,000 ppm (SOPP), with varying amounts of NaHCO3,in diet *ad libitum*, for 104 weeks. F344 rats. Males. 30 to 31 rats/group	SOPP is carcinogenic in rat urinary bladder, while OPP is not. Morphological changes of the bladder epithelium, correlating with increased urinary pH and Na^+ concentration.	Fukushima *et al.* (1989) (CA) B.6.8.2-04
12-week study.	OPP SOPP	2.0% SOPP for 64 weeks (experiment 1) 2.0% OPP for 64 weeks (experiment 2) SOPP : 0, 2500, 5000, 10,000, 20,000 ppm, ad libitum in diet for 36 weeks (experiment 3) F344 rats . Males. ~30/group.	Under the conditions of this study administration OPP after BBN treatment had no significant tumour-promoting activity whereas SOPP acted as a tumour promoter. At 20,000 ppm: morphological changes of the bladder luminal surface evident by SEM.	Fukushima *et al*. (1985) (CA) B.6.8.2-05
In-vitro metabolism of PHQ.	PHQ	0.2 mM PHQ incubated with 200 U PGHS.	PHQ can be metabolised *in-vitro* by PGHS yielding PBQ. Prostaglandins and the metabolism of araquidonic acid may play an important role in the detoxification processes of OPP and their metabolites.	Kolachana *et al*. (1991) (CA) B.6.8.2-06
In-vitro metabolism of PHQ and PBQ.	PHQ PBQ	0.05-0.5 M solution of PHQ or PBQ.	Autoxidation of PHQ to PBQ is accelerated when pH values increase. The presence of PBQ and O2 further accelerates this reaction.	Kwok & Eastmond (1997) (CA) B.6.8.2-07
Tumour initiation / promotion.	OPP SOPP	20,000 ppm OPP or SOPP, in the diet for 32-weeks. F344 rats. Males. 30/ group.	SOPP acts as a tumour promoter following initiation by BBN. SOPP alone also induced tumour formation and can therefore be considered a weak initiator. OPP had no significant tumour-promoting or -initiating effects.	Fukushima *et al*. (1983) (CA) B.6.8.2-08
Carcinogenicity study.	OPP SOPP	12,500 ppm (OPP), 20,000 ppm (SOPP), with/without NaHCO3 in the diet for 26 weeks. F344/DuCrj rats. Males. 31/group.	Urinary bladder tumorigenesis by OPP is enhanced by NaHCO3. Conversely, the carcinogenic potential of SOPP is reduced by co-administration of an acidifier, NH4Cl, which made it less potent than OPP.	Fujii *et al.* (1987) (CA) B.6.8.2-09
Carcinogenicity study.	OPP SOPP	20,000 ppm OPP or SOPP, dietary for 32-week. F344 rats. Males. 15/group.	Reduced urinary osmolality. Increased pH and Na+ correlate with tumorigenesis.	Fukushima *et al*. (1986) (CA) B.6.8.2-10

Type of study/data	Test substance	Relevant information about the study	Observations	Reference
Mechanistic, DNA-binding study.	OPP SOPP	(Short-term) OPP, SOPP: 2% in diet for 90-day. (Acute) OPP, SOPP: 500 mg/kg by gavage for 16 hours. F344 rats. Males. 30 or 8/group (short-term or acute).	SOPP, but not OPP, caused regenerative hyperplasia of the urinary bladder. OPP-treated rats revealed renal damage. No interactions with DNA could be demonstrated for either compound.	Reitz *et al*. (1983) (CA) B.6.8.2-11
Carcinogenicity study.	OPP SOPP	OPP: 1.25% with or without NaHCO3 SOPP: 2% with or without NH4Cl. In the diet for 8 weeks.	Males are more sensitive to OPP than females under alkalinuric conditions with respect to bladder hyperplasia.	Hasegawa *et al*. (1991) (CA) B.6.8.2-12
Carcinogenicity study.	OPP SOPP	OPP, SOPP: 0.1-2.0% dietary for 1-week (agglutination assay) or 50-weeks (in-vivo carcinogenesis experiment). F344 rats. Both sexes. 5 or 6 / group and sex.	OPP and SOPP caused a dose-dependent increase in agglutinability of bladder epithelial cells by Con A which is an indication for carcinogenic potential. SOPP caused carcinomas or preneoplasic lesions in urinary bladder and also but with lower incidence in renal pelvis of male rats.	Honma *et al*. (1983) (CA) B.6.8.2-13
Mechanistic	OPP PHQ PBQ	OPP, PHQ, PBQ: 700, 1400 mg/kg bw, single oral gavage, with or without inhibition of GSH synthesis. F344 rats. Males. 4 / group.	OPP treatment led to GSH depletion and liver and kidney damage. Inhibition of GSH synthesis aggravated hepatotoxicity of OPP. In addition, an intermediate of OPP (PBQ) induced hepatic and renal damage as well.	Nakagawa & Tayama (1988) (CA) B.6.8.2-14
In-vitro cytotoxicity test.	OPP PHQ	OPP, PHQ: 0–1 mM In male F344 rat hepato-cytes.	OPP cytotoxicity is enhanced by monooxygenase inhibition and GSH depletion. PHQ-induced cell death can be inhibited by sulfhydryl compounds.	Nakagawa *et al*. (1992) (CA) B.6.8.2-15
In-vitro metabolism of OPP and its metabolites.	OPP	OPP: 1-100 µM	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 (redox cycling).	Roy D.(1990) (CA) B.6.8.2-16
In-vivo assay of DNA synthesis in bladder.	OPP, SOPP	OPP, SOPP: 2% in diet; for 4–24 weeks. F344 rats. Males. 20 / group.	OPP and SOPP cause a proliferative response in renal pelvis and papilla when given at a dietary level of 2%.	Shibata *et al*. (1989) (CA) B.6.8.2-17
In-vitro and *in-vivo* GSH conjugation.	OPP	*In-vitro* study: 79 µg/mL *In-vivo* study: 1000 mg/kg, single oral dose. F344 rats. Males.	PHQ-GSH is excreted via the bile after OPP administration to rats. In-vitro, PHQ-GSH can be formed non-enzymatically from PBQ and GSH or enzymatically from OPP and GSH.	Nakagawa & Tayama (1989) (CA) B.6.8.2-18
In-vitro interaction with PGHS.	OPP PHQ PBQ	OPP, PHQ, PBQ: 100 µM.	OPP and PHQ stimulate cyclooxygenase activity and are oxidised by PGHS. OPP, PHQ and PBQ inhibit PGHS at higher concentrations.	Freyberger (1994) (CA) B.6.8.2-19

Type of study/data	Test substance	Relevant information about the study	Observations	Reference
Ten-week feeding study in rats.	OPP SOPP	OPP: 1.25% in diet SOPP: 2.0% in diet for 10 weeks. F344 rats . Males. 10 to 13 / group.	OPP and SOPP caused urothelial hyperplasia in rats as evident by histology and increased cell proliferation.	St. John *et al.* (2001) (CA) B.6.8.2-20
In-vitro and *in-vivo* macro-molecular binding assay.	OPP SOPP	14C-OPP: 1 µCi *In-vivo:* OPP, SOPP: 50-500 mg/kg, oral gavage, 16-18 h. F344 rats. Males. 4 / group.	A non-linear increase in macromolecular binding of OPP and SOPP was observed *in-vivo* and *in-vitro*. This may be caused by the saturation of detoxification pathways.	Reitz *et al.* (1984) (CA) B.6.8.2-21
In-vivo assay of DNA and protein adducts in rats.	OPP	*In-vivo:* 0, 15, 50, 125, 250, 500, 1000 mg/kg bw OPP, single oral gavage. F344 rats. Males.	OPP or its metabolites form protein, but not DNA, adducts in urinary bladder tissue.	Kwok *et al.* (1999) (CA) B.6.8.2-22
Enzyme induction study in mouse liver.	OPP	500, 1000 mg/kg bw/day OPP in the diet for 7 or 14 days. Males. B6C3F1 mice . 3 dose/time point.	Among the nuclear receptors AhR, CAR, PXR, and PPARα, only PPARα mediated gene expression was elevated following OPP exposure.	Geter *et al.* (2009) (CA) B.6.8.2-23
In-vitro PXR transactivation assay.	OPP	0.1 - 10 µM OPP.	OPP leads to transactivation of the human PXR, but not of the murine PXR.	Kojima *et al.* (2011) (CA) B.6.8.2/24

2.6.5.1 Short summary and overall relevance of the provided information on long-term toxicity and carcinogenicity

The notifier presented three dietary studies in rats and one in mice; additionally a 2-year dermal study in mice is available as well (See table 53).

Rats:

-Due to high number of deficiencies found in the first study (Hodge, 1952, B.6.5/01), limited information about long-term and carcinogenicity can be derived from it. In any case, 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 (Wahle & Christenson, 1996, 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. There was an increase in urinary bladder hyperplasia at 12 and 24 months in high dose males (and high dose females at 24 months) along with an increase in congestion, haemorrhage, mineralisation and necrosis. Non-neoplastic findings consisted on 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. In male rats there was an increased incidence of urinary bladder papillomas, transitional cell carcinomas, and/or combined papillomas and/or transitional cell carcinomas at 8000 ppm. The **NOAEL for systemic long-term toxicity was 800 ppm (39 mg/kg bw/day), the neoplastic NOAEL was 4000 ppm (200 mg/kg)**.

-The third study (Hiraga *et al*, 1984, B.6.5/03) is a published report. OPP was mixed with the diet at concentrations of 6500, 12500 or 25000 ppm to groups of 20-24 male F344 rats during 91 weeks to evaluate the carcinogenicity of OPP to the urinary tract. Under conditions of this study, OPP was carcinogenic in male F344

rats, causing urinary bladder tumours (papilloma and carcinoma) at 12500 ppm. Hyperplasia and calculi were also observed at 12500 and 25000 ppm. Increased mortality, decreased body weight and nephrotoxicity was also found at dose of 12500 and 25000 ppm. The **NOAEL (oncogenic and systemic) was established at 6250 ppm (269 mg/Kg/day).**

-Additionally, urothelial hyperplasia of the urothelium was detected in males in the first generational study in rats (Eigenberg, 1990, B.6.6.1-01), and in males and females in the second generational study (Eigenberg & Lake, 1995, B.6.6.1-02)(See table 55).

The **target organ for long-term toxicity of OPP in rats is the urinary bladder and, to a lesser extent, the kidney**. Dose- and time dependent hyperplasias and neoplasias of the urinary bladder epithelium were found.

Mice:

-In a dietary study (Quast & McGuirk, 1995, B.6.5-04), mice were administered 2-phenylphenol 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 males and females, a dose-related decrease of microvacuolation 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. 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. A data gap for historical control values was set by the experts. The **NOAEL for systemic toxicity in mice was <250 mg/kg bw/day, whereas the NOAEL for tumours was 250 mg/kg bw/day.**

-A 2-year dermal study (National Toxicology Program, 1986, 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 O-Phenylphenol caused non-neoplastic lesions, which included ulceration, inflammation, and hyperkeratosis, at the site of application. The **NOAEL for systemic toxicity was established at 55.5 mg/ animal/day** based on these non-neoplastic skin lesions at the application site.

In mice, the liver was the primary target organ affected by ingestion of OPP in male and female mice. The kidney was also affected, however only in males. Dietary OPP promotes tumour formation in hepatocytes, but not skin tumours when applied dermally, even after pre-treatment with a tumour initiator.

Except for the first study by Luster MI, *et al.* (1981, B.6.8.2-01) the remaining studies in section 2.6.8.2 (section B.6.8.2 in Volume 3 of this report) have been grouped under the umbrella of "**mechanistic studies**". They investigate the carcinogenic potential and MoA of OPP and SOPP, particularly in relation to rat urinary bladder tumours and mouse liver tumours (See table 55). The main conclusions from these studies are:

-The tumorigenic potential of OPP was enhanced by co-administration of sodium bicarbonate as an alkalinising agent, while the tumorigenesis of SOPP was attenuated by co-administration of ammonium chloride as an acidifier (Fujii *et al*., 1987, B.6.8.2-09; Hasegawa *et al.*, 1991, B.6.8.2-12). Besides pH, morphological changes of the bladder epithelium were also enhanced by reduced urinary osmolality (Fukushima *et al*., 1986, B.6.8.2-10), and increased Na+ concentration (Fukushima *et al*., 1989, B.6.8.2-04).

-Increased DNA synthesis in the bladder epithelium could be detected following OPP (Shibata *et al*., 1989, B.6.8.2-17) and SOPP (Klein, W., 1986, B.6.4.2.3-04) administration to rats. This mitotic activity could be clearly associated with morphological changes of the bladder epithelium (St John *et al*., 2001, B.6.8.2-20).

-In the 13-weeks study by Christenson, *et al*. (1996a, 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 a hyperplasia were seen in male rats.

-No DNA-adducts could be detected after treating rats with OPP or SOPP (Reitz *et al*., 1983, B.6.8.2-11). This is in accordance with observations made in a subchronic rat study (Christenson *et al*., 1996b, B.6.8.2-03), suggesting that bladder carcinogenesis is likely mediated by a cytotoxic rather than a genotoxic effect.

-However, *in-vivo* binding of OPP and SOPP to cellular macromolecules was described in one study, without specifying the nature of these macromolecules (Reitz *et al*., 1984, 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 (Roy D., 1990, B.6.8.2-16). See figure 2.6.5.2/1

-A later study on rats showed that OPP or its metabolites form protein adducts in the bladder, whereas DNA adducts could not be found (Kwok *et al*., 1999, B.6.8.2-22). The study also showed that the bladder has a greater tendency for protein adduct formation than liver and kidney, which could potentially be explained by an involvement of PGHS, an enzyme known to oxidise phenolic compounds to more reactive quinone species. This enzyme is highly expressed in the urinary bladder.

-Both OPP and PHQ 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 (Freyberger, 1994, B.6.8.2-19). The latter finding might explain the observations made in the 91-week-study on rats by Hiraga and Fujii (1984). In their study (B.6.5-03), an increased incidence of bladder tumours was seen at dietary OPP levels of 12,500 ppm, but not at 25,000 ppm.

-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 (Nakagawa & Tayama, 1988, B.6.8.2-14; Nakagawa, 1989, 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 (Nakagawa, 1992, B.6.8.2-15). See figure 2.6.5.2/1.

-Fukushima *et al.* (1983, B.6.8.2-08) investigated the tumour-promoting properties of OPP and SOPP after initiation with BBN. SOPP, when given via diet for 36 weeks at a concentration of 20,000 ppm, promoted tumour formation of the urinary bladder epithelium after initiation with BBN and was also weakly tumorigenic without prior initiation. However, OPP (20,000 ppm) alone did not cause neoplasias in the urothelium with or without initiation with BBN.

-Honma *et al*., 1983 (B.6.8.2-13) evaluated the bladder carcinogenicity of OPP and SOPP by a short-term assay for agglutinability of bladder epithelial cells with concanavalin A and investigated the carcinogenicity of SOPP in male rat, administered in diet for 50 weeks. OPP and SOPP caused a dose-dependent increase in agglutinability of bladder epithelial cells by Concanavalin A, indicative of carcinogenic potential and SOPP caused carcinomas or preneoplasic lesions in urinary bladder and also but with lower incidence in renal pelvis of male rats.

-Among the nuclear receptors AhR, CAR, PXR, and PPARα, only PPARα mediated gene expression was elevated following OPP exposure in mice (Geter *et al.*, 2009, B.6.8.2-23).

-OPP leads to transactivation of the human PXR, but not of the murine PXR (Kojima *et*, 2011, B.6.8.2/24).

So, in general a non-genotoxic **MoA for tumorigenesis in rat urinary bladders** is likely (Reitz *et al*., 1983, B.6.8.2-11; Christenson *et al*., 1996b, B.6.8.2-03). 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[1]; followed by regenerative hyperplasia and eventually tumours. Males seem to be more affected than females (Hasegawa *et al.*, 1991, B.6.8.2-12). Metabolism studies had showed than OPP in rodents is rapidly converted into conjugates which are eliminated via urine, the same can be applied to humans (Selim, S., 1996, B.6.1.2-01; Bartels, M. *et al.*, 1997, B.6.1.2-02). *In-vitro* genotoxicity studies performed with main 2-phenylphenol 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 in bladder 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

The **MoA for liver adenomas in mice** (found in Quast & McGuirk, 1995, B.6.5-04) seems to involve PPARα-dependent rodent liver tumour response (Geter *et al.*, 2009, B.6.8.2-23; Kojima *et*, 2011, B.6.8.2/24)**.**

Ortho-Phenylphenol classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). Classification regarding carcinogenicity is not included.

2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

According to CLP criteria (Regulation (EC) No 1272/2008), a carcinogen is a substance or a mixture that induces cancer or increases its incidence. Substances that have induced benign and malignant tumours in well-performed

[1] Available at URL: https://onlinelibrary.wiley.com/doi/pdf/10.1002/3527600418.mb9043verd0060 (accessed 06 May 2019)

experimental studies on animals are also considered to be presumed or suspected human carcinogens, unless there is strong evidence that the mechanism of tumour formation is not relevant for humans.

Some important factors, which may be taken into consideration, when assessing the overall level of concern, are:

(a) tumour type and background incidence;

(b) multi-site responses;

(c) progression of lesions to malignancy;

(d) reduced tumour latency;

(e) whether responses are in single or both sexes;

(f) whether responses are in a single species or several species;

(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;

(h) routes of exposure;

(i) comparison of absorption, distribution, metabolism and excretion between test animals and humans;

(j) the possibility of a confounding effect of excessive toxicity at test doses;

(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.

Chronic toxicity/carcinogenicity studies with OPP were conducted in two species (rats and mice).

Urinary bladder tumours in rats

In **rats**, **urinary bladder tumours** were seen in males at doses of 402 mg/kg bw/day. The following points argued by the applicant seem to suggest that the MoA for bladder carcinogenesis is specific to the rat:

-OPP has been shown to act as a tumour promoter only, not as a tumour initiator (Fukushima *et al*., 1983, B.6.8.2-08).

-Protein- but no DNA-binding of OPP metabolites has been detected in the urinary bladder (Christenson *et al*., 1996b, 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.

-As can be seen in figure 2.6.5.2/1, 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 (Reitz *et al.*, 1984, B.6.8.2-21).

-Increased urinary pH and sodium concentration promote bladder neoplastic effects by OPP (Fukushima *et al*., 1986, B.6.8.2-10; Fukushima *et al.*, 1989, 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.

These factors seem to suggest that the MoA that causes these tumours after OPP exposure is specific to the rat and not relevant for humans, however:

-OPP has been shown to act as a tumour promoter only, not as a tumour initiator

-Protein- but no DNA-binding of OPP metabolites has been detected in the urinary bladder, However UDS has been detected after SOPP treatment (Klein, W., 1986, B.6.4.2.3-04). Moreover PBQ (an OPP metabolite present in rats) caused DNA damage in the urinary bladder epithelium (Morimoto, K., *et al*., 1987, B.6.4.2.3-01).

-Although neoplasias have not been detected in the urinary bladder of female rats, hyperplasias of the urothelium have (Eigenberg, 1990, B.6.6.1-01; Eigenberg & Lake, 1995, B.6.6.1-02).

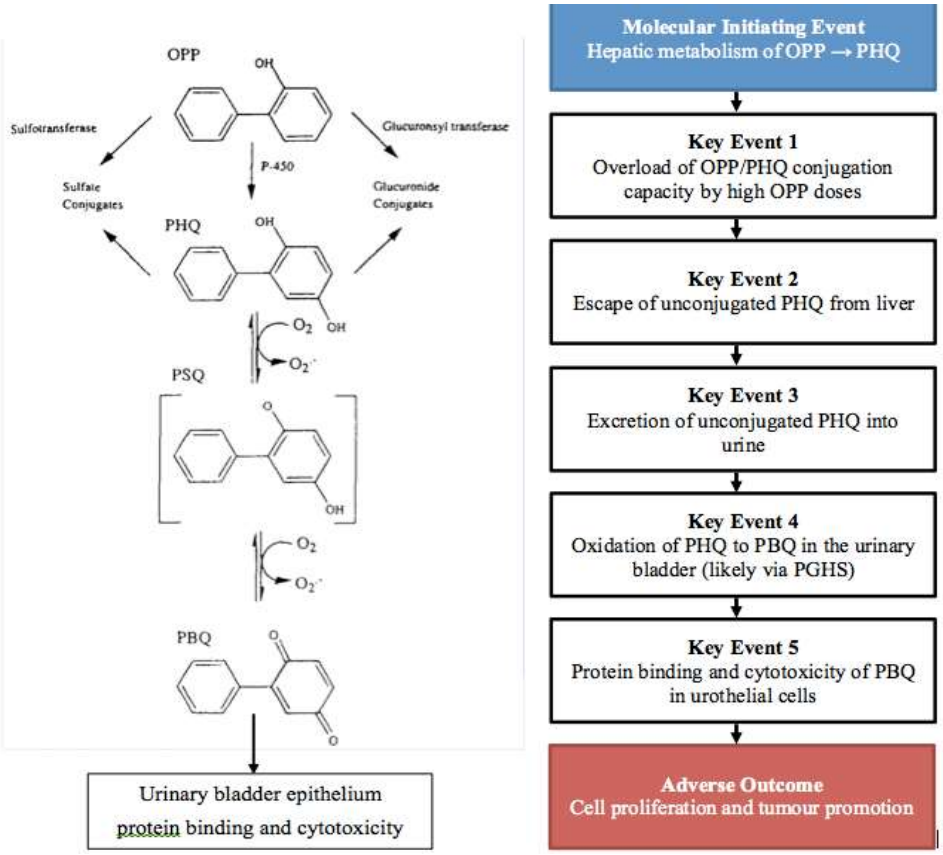
-The fact that high systemic OPP doses are necessary to elicit this effect bears no relevance as to the specificity of this MoA to the rat. Moreover, human absorption and distribution of OPP/SOPP is similar to that of rats (Selim, S., 1996, B.6.1.2-01; Bartels, M. *et al.*, 1997, B.6.1.2-02)

-The fact that the pH and sodium concentration of human urine is lower than in rat urine does not make the suggested MoA rat specific either.

-An effect may only occur in one animal model and still be relevant to humans. So in summary, even though a quite plausible non-genotoxic mechanism has been postulated, the MoA for rat bladder tumours remains in essence unknown and aneugenicity has not been adequately addressed *in vivo*. Thus **the relevance of the mechanism for humans cannot not be excluded.**

According to the criteria contained in Regulation (EC) No. 1272/2008, and in the absence of human studies, to classify a substance as a carcinogen in category 1, **sufficient evidence**[2] of carcinogenicity in animal studies is necessary. However bladder tumours appeared only in rats and only in males, which the RMS considers only as **limited evidence**[3]**.** Hence, according Regulation (EC) No. 1272/2008, **OPP** should be classified in **category 2**.

Figure 2.6.5.2/1 Adverse Outcome pathways for bladder carcinogenesis



Liver adenomas in mice:

Statistically significant increase of liver adenomas was described in 2-year mice study (Quast & McGuirk, 1995, B.6.5-04) for mid and high dose male groups (80% and 82%, respectively), compared to controls (54%). Although suitable historical control data were not available at the performing laboratory for the B6C3F1 strain of mouse, the National Toxicology Program (NTP) has extensive historical control data for this strain of mouse[4] during period 1990-1997. In addition, 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%).

[2] CLP defines sufficient evidence as: "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. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence."

[3] "the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs. "

[4] Haseman JK, Hayle JR, and Morris RW. 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.

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 conducted by Quast and McGuirk also combined these three type of tumours to show a statistically significant increase in mid and high dose groups (90% and 86%, respectively) compared to controls (64%). However, hepatoblastomas originate from a different cell population and adding these tumors to hepatocellular adenomas and carcinomas is not an appropriate method to determine statistical significance of liver tumors[5].

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

The MoA for liver neoplams 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 (Geter *et al.*, 2009, B.6.8.2-23)**.** However, RMS deems that more experimental evidences are needed to suggest a plausible MoA (e.g., PPARα activation, hepatocyte proliferation and apoptosis assays, or modulating factors such as oxidative stress or NF-κB activation). It is known that the PPAR-dependent rodent liver tumor response is "not relevant" or "unlikely to be relevant" to humans[5], as explicitly mentioned in the OECD guidance for analysis and evaluation of chronic toxicity and carcinogenicity studies [ENV/JM/MONO(2002)19].

Thus, taken all together, and based on the available data, there are no evidences of liver carcinogenity after OPP administration. Moreover, the fact that mice hepatocellular adenomas were only increased in male mice, but no in female mice or in rats.

Table 56: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Rat.	Urinary bladder papillomas and transitional cell carcinomas. Background incidence in males in this lab: -Papillomas from 1/409 to 1/503. -Carcinomas from 2/409 to 2/50.	No.	Yes.	-	Only males.	No	Diet.	Unknown, likely non-genotoxic. **Relevance to humans cannot be discarded.**
Mouse.	Hepatocellular adenoma, and hepatoblastoma (nearly located within a pre-existing adenoma). High background incidence (54% in controls, 82% in the high-dose	Leydig cell tumours were found in 2 male mice at the highest dose and in 1 in the control group, however they are thought to	No.	-	Only males.	No.	Diet.	PPARα-dependent rodent liver tumour response. **Irrelevant.**

[5] Corton JC, Peters JM, Klaunig JE. The PPARα-dependent rodent liver tumor response is not relevant to humans: addressing misconceptions. *Arch Toxicol*. 2018;92(1):83-119. doi:10.1007/s00204-017-2094-7.

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
	group.	be unrelated to treatment.						

2.6.5.3 Conclusion on classification and labelling for carcinogenicity

Based on the data available for ***ortho*-phenylphenol (OPP)**, and according to the criteria under Regulation (EC) No. 1272/2008, RMS proposes the classification of this active substance as **carcinogenic in category 2 (H351).**

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)*

		Number of rats with:			
OPP [ppm]	**Examined rats**	**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[1]. 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.

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,

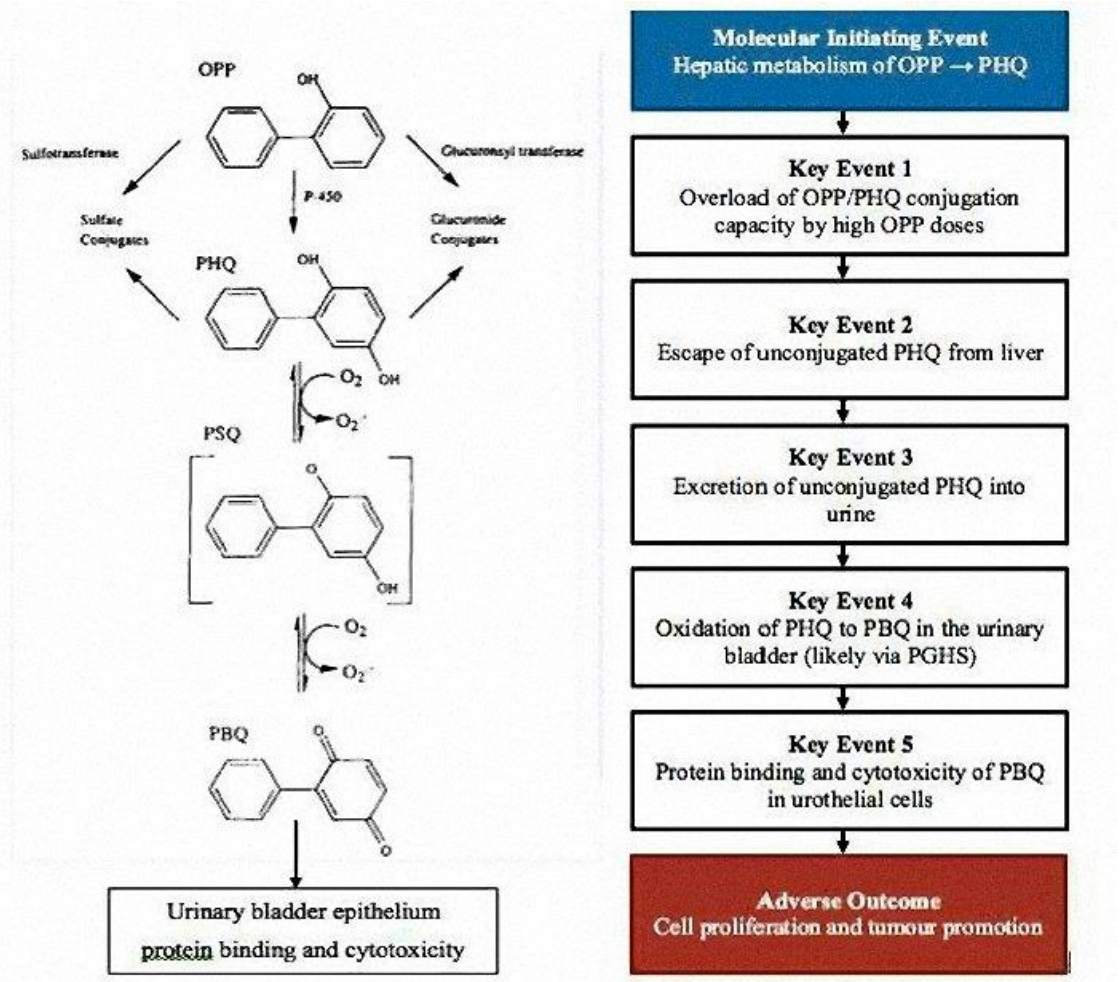
[1] Haseman *et al.*, Spontaneous Neoplasm Incidences in Fischer 344 Rats and B6C3F, Mice in Two-Year Carcinogenicity Studies: A National Toxicology Program Update*Toxicol Pathol., 1998, 26(3):428-41.

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.

2.6.6 Summary of reproductive toxicity [equivalent to section 10.10 of the CLH report template]

Toxicology database of OPP is extensive, but the main focus is their carcinogenicity and the associated mode-of-action (MOA). To illustrate, in the past two decades, over 90 studies in the open literature investigated these aspects of OPP toxicity. By contrast, there are only seven reports on their developmental or reproductive effects.

2.6.6.1 Adverse effects on sexual function and fertility – generational studies [equivalent to section 10.10.1 of the CLH report template]

Table 57: Summary table of animal studies on adverse effects on sexual function and fertility – generational studies

Method **Guideline.** **Deviations if any.** **Acceptability** **Species, strain** **Sex** **No/group**	**Test substance.** **Route of expousure** **Dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL** **[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)]**	**Reference**
Two-generation, rat OECD 416. Deviations: Dose spacing and resting period before the second mating lasted longer than recommended. Cohousing period was shorter than recommended. No assessment of sexual maturation, sperm parameters, corpora lutea, and uterine implantation sites was performed. Some organ	OPP (purity 99.86%) 40, 140 and 490 mg/kg bw/day (Actual doses: 35, 125, 457 mg/kg bw/day)* administered in the diet for two generations(All animals (except for two high dose group F1 females and twelve F2A pups) were exposed to the test compound from initiation of the study until scheduled sacrifice). Study scheme P →F1A and F1B	**PARENTAL ANIMALS** *Mortality* ***P:*** • 2 control ♀ died, one on day 24 of gestation and the other on week 24 , both due to undetermined causes. • 1 control ♀ was terminated on gestation day 24 due to dystocia. • 2 ♀ (40 mg/kg bw/day) were terminated on weeks 5 (due to malocclusion) and 31 (due to pale eyes and a mass on the front leg). • 1 ♀ (140 mg/kg bw/day) died on week 14 due to treatment effects resulting mainly in severe urinary bladder transitional cell hyperplasia and calculi formation. • 1 ♂ (40 mg/kg bw/day) died on week 16 due to chronic kidney disease and abdominal haemorrhage. • 2 ♂ (140 mg/kg bw/day) died on weeks 26 and 36 due to malignant lymphoma and undetermined causes respectively. • 2 ♂ (140 mg/kg bw/day) were terminated on week 14 and 24 due to inferior brachygnathia and malocclusion respectively. • 1 ♂ (490 mg/kg bw/day) was terminated on week 5 due to inferior brachygnathia.	Eigenberg (1990) (CA) B.6.6.1-01

Method Guideline. Deviations if any. Acceptability Species, strain Sex No/group	**Test substance. Route of expousure Dose levels, duration of exposure**	**Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL [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)]**	**Reference**
weights were not reported. **Accepted.** Albino CD Sprague-Dawley rats. Both sexes. At 25-35 per sex and dose group.	F1 →F2A and F2B F2 Range-finding study/ies: subchronic studies in which doses of 500 mg/kg produced clear toxicity while 50 mg/kg did not. **Mean concentrations as % of nominal concentrations were:* *40 mg/kg/day: ♂ 87.8%, ♀ 89.6%* *140 mg/kg/day: ♂ 90.6%, ♀ 87.4%* *490 mg/kg/day: ♂ 92.5%, ♀ 93.9%*	***F1:*** • 1 ♀ (140 mg/kg bw/day) died on gestation day 22 due to undetermined causes. • 1 ♀ (490 mg/kg bw/day) died on gestation day 22 due to undetermined causes. • 1 ♂ (40 mg/kg bw/day) died on week 70 due to undetermined causes. • 2 ♂ (140 mg/kg bw/day) were terminated on weeks 61 and 70 both due to weight loss. • 2 ♂ (490 mg/kg bw/day) were terminated on weeks 46 and 61 due to inferior brachygnathia and malocclusion. **490 mg/kg bw/day** ***P:*** ▪ ↓ bw in ♂/♀ during pre-mating [week 5 (5%/-), week 7 (-/5%), week 8 (7%/7%), week 9 (6%/6%) ,week 10 (6%/7%), week 11 (-/8%), week 11 (-/8%), week 12 (8%/7%), week 13 (8%/7%), week 14 (8%/8%) and week 15 (8%/-)] and in ♀ during gestation of F1A/F1B [GD 0 (7%/10%), GD 6 (4%/8%) and GD 13 (-/7%)] and lactation of F1A [LD 4 (7%) and LD 7 (6%)]. ▪ ↓ terminal bw in ♂/♀ (6%/12%) ▪ ↓ bw gain in ♂/♀ [at week 15 of pre-mating (23%/24%)] and ↑ in ♀ during gestation of F1A [at day 21 (21%)]. ▪ ↓ feed consumption in ♂/♀ during pre-mating [week 2 (-/6.7%, ndr), week 8 (15%/11%), week 11 (7%/-), week 12 (7%, ndr/-), week 15 (14%/15%), week 27 (-/11%), week 28 (-/11%), week 29 (-/10%), week 30 (-/15%) and week 31 (-/14%)] as well as ↑ feed consumption in ♂ [week 9 (15%/14%)]. ▪ ↑ rel. wt. of ovaries in ♀ (33%, ndr) and of kidney in ♂ (7%). ▪ ↑ incidence of renal calculus (13/35 Vs. 3/35 in controls) and haemorrhage (6/35 Vs. 0/35 in controls) in ♂. ▪ ↑ incidence of urinary bladder transitional cell hyperplasia in ♂ (23/35 Vs. 3/35 in controls) and ♀ (9/35 Vs. 1/35) ▪ ↑ incidence in average no. cells/layer 81% in ♂ and 32% in ♀. ↑ of average microns at 10X 142% in ♂ and 50% in ♀. ***F1:*** ▪ ↓ bw in ♂/♀ during pre-mating [week 42 (12%, ndr/-), week 43 (10%, ndr/-), week 44 (70%/-), week 45 (9%, ndr/-), week 46 (11%/7%), week 47 (10%/7%), week 48 (11%/8%), week 49 (12%/-), week 50 (12%/9%), week 51 (11%/9%) and week 52 (12%/11%)] and in ♀ during gestation of F2A/F2B [GD 0 (8%//9%), GD 6 (3%, ndr/7%) and GD 13(9%/8%)] and lactation of F2A/F2B [LD 0 (6%/-), LD 4 (10%/11%%), LD 7 (6%/8%) and LD 14 (-/8%, ndr)]. ▪ ↓ terminal bw in ♂/♀ (11%, ndr/10%). ▪ ↓ bw gain in ♂/♀ [at week 52 of pre-mating period (13%/20%) and ↑ in ♀ during gestation of F2B [at day 21 (20%, ndr)]. ▪ ↓ feed consumption in ♂/♀ [week 44 (8%, ndr/-), week 45 (10%, ndr/-), week 46 (7%, ndr/-), week 48 (9%, ndr/-), week 49 (7%, ndr/-), week 50 (10%, ndr/-), week 52 (9%, ndr/-) and week 64 (10%/9%)]. ▪ ↓ abs. wt. of liver (13%) and kidney (9%) in ♀	

Method Guideline. Deviations if any. Acceptability Species, strain Sex No/group	Test substance. Route of expousure Dose levels, duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL [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)]	Reference
		▪ ↑ rel. wt. of testes (13%) and kidney (11%) in ♂ ▪ ↑ incidence of urinary bladder transitional cell hyperplasia in ♂ (15/35 Vs. 1/27 in controls). ▪ ↑ of average microns at 10X 62% in ♂ **<u>140 mg/kg bw/day</u>** ***P:*** ▪ ↑ in bw gain ♀ during gestation of F1A [at day 21 (20%)] ▪ ↓ feed consumption in ♂/♀ during pre-mating [week 8 (18%/15%), week 15 (14%/11%), and week 28 (-/7%)] as well as ↑ feed consumption in ♂/♀ [week 9 (10%/14%)]. ▪ ↑ incidence of average no. cells/layer 29% in ♀. ↑ of average microns at 10X 48% in ♂ and 51% in ♀. ***F1:*** ▪ ↑ abs. wt. of liver (10.3%, ndr), kidney (9%, ndr) and testes (8%, ndr) in ♂. ▪ ↓ incidence of average no. cells/layer 26% in ♀ (ndr). **<u>40 mg/kg bw/day</u>** ***P:*** There were no treatment-related effects. ***F1:*** ▪ ↓ bw in ♂ during pre-mating [week 42 (14%, ndr), week 43 (10%, ndr), week 44 (9%), week 45 (6%, ndr) and week 46 (5%)] . ▪ ↓ feed consumption in ♂ (week 43 (7%, ndr). ▪ ↑ abs. wt. of kidney (7%, ndr) and testes (6%, ndr) in ♂. **REPRODUCTIVE PARAMETERS** ***P and F1*** **<u>490 mg/kg bw/day</u>** ▪ ↑ ♀ fertility index (47%, ndr; ns.) during F1b generation vs 32% in controls. **<u>140 mg/kg bw/day</u>** ▪ ↑ ♀ fertility index (64%, ndr) during F1b generation vs 32% in controls. **<u>40 mg/kg bw/day</u>** ▪ ↑ ♀ fertility index (68%, ndr) during F1b generation vs 32% in controls. **LITTER DATA** **<u>490 mg/kg bw/day</u>** ***<u>P→F1A and F1B</u>:*** ***P→F1A*** ▪ ↑ live birth index (12%). 100% vs 88% in controls. ***P→F1B*** ▪ ↓ Pup bw. [day 14 (13%) and day 21 (18.4%) *post partum*]. ***<u>F1→F2A and F2B</u>*** ***F1→F2A*** ▪ ↓ Pup bw. [day 14 (6%) and day 21 (12%) *post partum*]. ***F1→F2B*** ▪ ↓ Pup bw. [day 21 (12%) *post partum*].	

<table>
<tr><th>Method
Guideline.
Deviations if any.
Acceptability
Species, strain
Sex
No/group</th><th>Test substance.
Route of expousure
Dose levels, duration of exposure</th><th>Results
- NOAEL/LOAEL (for sexual function and fertility, parents)
- target tissue/organ
- critical effects at the LOAEL

[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)]</th><th>Reference</th></tr>
<tr><td></td><td></td><td><u>140 mg/kg bw/day</u>

<u>P→F1A</u>:
▪ ↑ live birth index (9%). 97% vs 88% in controls
▪ ↑ 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.

Table 1:Summary of pelvis dilatation (kidney) on F1 and F2 pups (21 days or older)

<table>
<tr><th></th><th colspan="4">F1a males</th><th colspan="4">F1b males</th></tr>
<tr><th>Parameter \ Dosage</th><th>0</th><th>40</th><th>140</th><th>490</th><th>0</th><th>40</th><th>140</th><th>490</th></tr>
<tr><td>Kidney: dilatation, pelvis</td><td>2/4 (50%)</td><td>5/6 (83.3%)</td><td>6/9 (54%)</td><td>3/6 (50%)</td><td>0 (0%)</td><td>2/2 (50 %)</td><td>2/2 (100 %)</td><td>1/4 (25%)</td></tr>
<tr><td></td><td colspan="4">F1a females</td><td colspan="4">F1b females</td></tr>
<tr><td>Kidney: dilatation, pelvis</td><td>8/9 (89%)</td><td>11/12 (91%)</td><td>12/13 (92.3%)</td><td>8/8 (100%)</td><td>0 (0%)</td><td>7/7 (10 0%)</td><td>3/3 (100 %)</td><td>0 (0%)</td></tr>
<tr><td></td><td colspan="4">F2a males</td><td colspan="4">F2b males</td></tr>
<tr><td>Kidney: dilatation, pelvis</td><td>0/3 (0%)</td><td>5/5 (100%)</td><td>1/1 (100%)</td><td>5/15 (33.3%)</td><td>1/4 (25%)</td><td>7/9 (77. 8%)</td><td>8/11 (72.7 %)</td><td>4/6 (66.7 %)</td></tr>
<tr><td></td><td colspan="4">F2a females</td><td colspan="4">F2b females</td></tr>
<tr><td>Kidney: dilatation, pelvis</td><td>4/8 (50%)</td><td>14/16 (87.5%)</td><td>19/19 (100%)</td><td>20/26 (76.9%)</td><td>2/8 (25%)</td><td>15/1 7 (88. 2%)</td><td>14/15 (93.3 %)</td><td>4/5 (80%)</td></tr>
</table>

-Parental LOAEL = 125 mg/kg bw/day
-Parental NOAEL = 35 mg/kg bw/day
-Critical effect at the LOAEL: bladder calculi (♂), urothelial hyperplasia (♂,♀)

-Offspring LOAEL = 457 mg/kg bw/day
-Offspring NOAEL = 125 mg/kg bw/day
-Critical effect at the LOAEL: calculi in kidney and bladder, renal damage, ↓ bw starting week 2 of lactation

-Reproductive LOAEL = > 457 mg/kg bw/day
-Reprodructive NOAEL ≥ 457mg/kg bw/day
-Critical effect at the LOAEL: n/a

-Target organs/tissues: Kidneys</td><td></td></tr>
<tr><td>Two-generation, rat
OECD 416.
Deviations: Same as in the previous 2-generation study by Eigenberg (1990), except dams were cohoused for appropriate</td><td>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 ♂/♀). P and F1 adults received OPP in the diet throughout the entire study. After</td><td>PARENTAL ANIMALS

Mortality

P:
• 1 ♂ (500 mg/kg bw/day) died on day 173 due to kidney failure.
• 2 ♀ (500 mg/kg bw/day) died on days 173 and 174 both due to dystocia.
• 1 ♂ (500 mg/kg bw/day) was terminated on day 168 due to upper respiratory tract infection.
• 2 ♀ (100 mg/kg bw/day) died on days 1 and 170 due to</td><td>Eigenberg & Lake (1995) (CA) B.6.6.1-02</td></tr>
</table>

Method Guideline. Deviations if any. Acceptability Species, strain Sex No/group	**Test substance. Route of expousure Dose levels, duration of exposure**	**Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL [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)]**	**Reference**
amounts of time. **Accepted.** Albino CD Sprague-Dawley rats. Both sexes. 30/sex/dose.	receiving the test compound for ten weeks, P adults were mated to produce F1a and F1b litter and F1 adults Study scheme F0 →F1A and F1B F1 →F2A and F2B F2 Range-finding: dose levels were selected based on the previous two-generation reproduction study	ruptured liver and dystocia respectively. • 1 ♀ (20 mg/kg bw/day) was terminated on day 176 due to dystocia. • 1 control ♀ was terminated on day 120 due to dystocia. ***F1:*** • 2 ♂ (500 mg/kg bw/day) were terminated on days 176 and 153 both due to malignant lymphoma. • 1 ♂ (20 mg/kg bw/day) died on day 14 due to undetermined causes. • 1 ♂ (20 mg/kg bw/day) was terminated on day 157 due to undetermined causes. **500 mg/kg bw/day** ***P:*** ▪ ↓ bw in ♀ during pre-mating [day 21(6%), day 28 (6%), day 42 (5%), day 49 (6%), day 56 (6%), day 63 (7%) and day 70 (7%), all ncdr], ↑ bw in ♂ during pre-mating on day 0 (4%, ndr) ↓ bw in ♀ during gestation of F1A/F1B [GD 0 (8%/7%), GD 6 (6%/8%), GD 13 (6%/8%) and GD 20 (5/7%), all ncdr] and ↓ bw in ♀ during lactation of F1A /F1B [LD 0 (8%/8%), LD 4 (7%/8%), LD 7 (8%/8%), LD 14 (8%/-) and LD 21 (8%/-)all ncdr]. ▪ ↓ terminal bw in ♀ [day 176 (8%)] ▪ ↓ food consumption in ♂ in week 7 (8%), ↑ food consumption in week 42 (5%), week 70 (6%), week 119 (5%), week 126 (8%), week 133 (11%), week 140 (9%). ↓ food consumption in ♀ in week 7 (6%). ↑ food consumption in week 42 (9%), week 49 (7%), week 56 (10%), week 63 (6%). ▪ ↑ incidence of histopathological alterations in ♂: in the urinary bladder [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)], and the ureter [dilatation (4/30 vs. 0/30 in controls) and hyperplasia (3/30 vs. 0/30 in controls)]. ***F1:*** ▪ ↓ bw in ♂/♀ during pre-mating [all weekly measurements (from day o to 175 in ♂ and from day 0 to 70 in females) show statistically significant bw reductions between 8 and 13% with no apparent trend in time or sex effect, and not present at any other dose level], ↓ bw in ♀ during gestation of F2A/F2B [GD 0 (7%/9%), GD 6 (-/11%), GD 13 (8%/10%) and GD 20 (7/8%), all ncdr] and ↓ bw in ♀ during lactation of F2A /F2B [LD 0 (8%/8%), LD 4 (7%/8%), LD 7 (8%/8%) and LD 14 (8%/-), all ncdr]. ▪ ↓ terminal bw in ♂ [day 152 (11%)] ▪ ↑ food consumption in ♂ in week 42 (7%), week 49 (7%), week 56 (11%), week 63 (10%), week 70 (12%). ↑ food consumption in ♀ in week 14 (8%), week 63 (8%), week 70 (7%). ▪ ↑ rel. wt. of testes (12%). ▪ ↑ incidence of histopathological alterations in ♂: in the urinary bladder [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)], and kidney [debris (4/30 vs. 0/30 in	

Method Guideline. Deviations if any. Acceptability Species, strain Sex No/group	Test substance. Route of expousure Dose levels, duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL [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)]	Reference
		controls)]. **100 mg/kg bw/day** ***P:*** There were no treatment-related effects except: ▪ ↓ food consumption in ♂ in week 14 (3%) (ndr), week 21 (7%) (ndr), week 28 (3%) (ndr). ↑ food consumption in ♂ in week 126 (4%) (ndr). ***F1:*** There were no treatment-related effects except: ▪ ↓ food consumption in ♀ in week 42 (5%) (ndr). **20 mg/kg bw/day** ***P:*** There were no treatment-related effects. ***F1:*** There were no treatment-related effects. **REPRODUCTIVE PARAMETERS** ***P,-F1 and F2*** **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%). *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 bw. [day 21 (12%)]. ***P→F1B*** ▪ ↓ Pup bw. [day 21 (10%)]. ***F1→F2A and F2B*** ***F1→F2A***	

Method Guideline. Deviations if any. Acceptability Species, strain Sex No/group	Test substance. Route of expousure Dose levels, duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL [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)]	Reference
		▪ ↓ Pup bw. [day 14 (6%) and day 21 (11%)]. . ***$F_1 \rightarrow F_{2B}$*** ▪ ↓ Pup bw. [day 14 (7%) and day 21 (12%)]. -**Parental LOAEL** = 459/457 (♂/♀) mg/kg bw/day -**Parental NOAEL** = 93/92 (♂/♀) mg/kg bw/day -**Critical effect at the LOAEL**: ↓ bw (♂,♀) and histopathology of the urinary bladder(♂) -**Offspring LOAEL** = 457 mg/kg bw/day -**Offspring NOAEL** = 92 mg/kg bw/day -**Critical effect at the LOAEL**: ↓ bw (♀) Decrease in pup bodyweight. -**Reproductive LOAEL** = > 459/457 (♂/♀) mg/kg bw/day -**Reproductive NOAEL** ≥ 459/457 (♂/♀) mg/kg bw/day -**Critical effect at the LOAEL**: n/a -**Target organs/tissues**: Urinary bladder/urothelium	

Table 58: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 59: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility – generational studies

The reproductive toxicity of OPP was assessed in two 2-generation rat reproductive studies. No generational studies with SOPP are available.

Since the original submission, the notifier has submitted an additional publication by Kwok and Silva (2013, B.6.6.2-6). This publication has been instrumental in the assessments of OPP/SOPP reproductive toxicity, and the RMS considers that the possibility that OPP/SOPP might be toxic for reproduction requires a re-evaluation that this assessment report aims to start.

-In the first two-generation study (Eigenberg, 1990, 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.

Regarding reproductive parameters, no differences were detected between treated groups and controls in both generations. Only female fertility index was increase in low and mid dose groups (68% and 64%, respectively) in F1b generation compared with controls (32%). However, this increase in the fertility index is considered an artifact 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).

Kidneys appeared to be the target organs. Relative kidney weights were statistically higher in 490 mg/kg bw/day P and F1 males. At the top dose, macroscopic alterations consisted of an increased incidence of calculus in kidneys and urinary bladder. Microscopically, an increased incidence of hyperplasia of transitional cells was observed in the urinary bladder, this increase was statistically significant in P and F1 males and P females treated with 457 mg/kg bw/day.

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 on the matter 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.

In this study, a **parental NOAEL of 40 mg/kg bw/day (Actual dose: 35 mg/kg bw/day)** and an **offspring NOAEL of 140 mg/kg bw/day (Actual dose: 125 mg/kg bw/day)** were established. The **reproductive NOAEL was ≥ 490 mg/kg bw/day (Actual dose: 457 mg/kg bw/day)** although it may have been derived from unreliable data (see sections B.6.6.1-01 and B.6.6.2-06 in volume 3) and deserves further discussion.

-In the second two-generation study (Eigenberg & Lake, 1995, 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 ♂/♀).

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 BW depression in F1 pups around the weaning period and earlier, from day 14 onwards in case of F2 offspring.

No effect on reproductive parameters was seen at any dose level. Although some parameters were not evaluated, such as sperm parameters and sexual maturation milestones. Another problem with reproductive parameters is the fact that the least ability to procreate (as indicated by the fertility index) was seen in F2a and F2b controls (as indicated by Kwok *et al*. in 2013, 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.

So similarly to the previous generational study by Eigenberg from 1990 (B.6.6.1-01), the assessments on fertility in this study are somehow unreliable.

The **parental and offspring NOAEL was 100 mg/kg bw/day (actual dose: 93/92 mg/kg bw/day, m/f).** The **reproductive NOAEL was ≥ 500 mg/kg bw/day (actual dose: 459 / 457 mg/kg bw/day, m/f)** although once again may have been derived from unreliable data (see sections B.6.6.1-02 and B.6.6.2-06) and should be the subject of further discussion.

Overall reproductive parameters were seemingly not affected in rats. Kidneys and urinary bladder were the target organs, where hyperplasia of the transitional epithelium cells and chronic inflammation were seen. The **overall parental and offspring NOAEL were established at 100 mg/kg bw/day (actual dose: 93/92 mg/kg bw/day, m/f)**, and the **reproductive NOAEL was 500 mg/kg bw/day (actual dose: 459 / 457 mg/kg bw/day, m/f)**.

Ortho-Phenylphenol classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). Classification regarding sexual function and fertility is not included.

2.6.6.1.2 Comparison with the CLP criteria regarding adverse effects on sexual function and fertility

For the purpose of classification for reproductive toxicity according to the criteria of the CLP (Regulation (EC) No 1272/2008), substances are allocated to one of two categories. Within each category, effects on sexual function, fertility, lactation and development, are considered separately

Category 1: Known or presumed human reproductive toxicant

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

Category 1A: Known human reproductive toxicant

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

Category 1B: Presumed human reproductive toxicant

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

Category 2: Suspected human reproductive toxicant

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

No human information is available on the effects of OPP on the reproductive system. Information from reliable two-generation studies in rats showed that OPP has no effects on sexual function and fertility. Consequently, classification is not warranted.

2.6.6.2 Adverse effects on development [equivalent to section 10.10.4 of the CLH report template]

Table 60: Summary table of animal studies on adverse effects on development

Method **Guideline.** **Deviations if any/Acceptability** **Species, strain** **Sex** **No/group**	**Test substance.** **Route of expousure** **Dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL** **[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)]**	**Reference**
Developmental toxicity, rat No guideline. **Supportive only** Wistar strain Rat. Females. 11 to 20 / Dose group.	OPP (purity 99.7%) Oral gavage 0, 150, 300, 600, 1200 mg/kg bw/day from day 6 to 15 (inclusive) of presumed gestation.	***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, pregnant rats fell into ataxia for several hours the severity of which was dose-dependent **600 mg/kg bw/day:** ▪ ↓ bw gain [(GD 9 (60%), GD 12 (51%), GD 15 (62%) and GD 20 (46%)]. **300 mg/kg bw/day:** ▪ ↓ bw gain [(GD 9 (17%), GD 12 (18%), GD 15 (28%) and GD 20 (20%)]. ***Developmental toxicity***	Kaneda *et al.* (1978) (CA) B.6.6.2/01

Method **Guideline.** **Deviations if any/Acceptability** **Species, strain** **Sex** **No/group**	**Test substance.** **Route of expousure** **Dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL** **[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)]**	**Reference**
		600 mg/kg bw/day: ▪ ↑ percentage of fœtal death (85%) ▪ ↓ mean fœtal weight in ♂/♀ (6%/8%) ▪ ↑ foetal incidence of malformations: o Cranial or sacral meningocele (1/237, 0.4%, ns) o Hydronephrosis (14/119, 11.8%, ns) o Diaphragmatic hernia) (1/119, 0.8%, ns) o Omphalocele (1/188, 0.8%, ns) **300 mg/kg bw/day:** ▪ ↑ foeatal incidence of malformations: o Cranial or sacral meningocele (2/188, 1.7%) ns) o Hydronephrosis (7/97, 7.2%, ns) o Diaphragmatic hernia (2/97, 2.1%, ns) -**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)	
Developmental toxicity, rat No guideline. **Supportive only** SD-Rat. Females. 25 to 35 / Dose group.	OPP (purity 99.69%) Oral gavage 0, 100, 300, 700 mg/kg bw/day, from day 6 to 15 (inclusive) of presumed gestation.	***Maternal toxicity*** *Mortality:* 1/25 (Vs. 0/35 in controls) dams died due to an accident during administration of the test substance **700 mg/kg bw/day:** ▪ ↓ bw [day 10 (6%) and day 16 (6%)] ▪ ↓ bw. gain [(days 6 to 9 (64%)]. ▪ ↓ abs. liver wt. [(days 21(18%)]. ***Developmental toxicity*** **700 mg/kg bw/day:** ▪ ↑Incidence of post-implantation loss: o Foetuses: 13.4% o Litters: 15/20 75% ▪ *Skeletal alteration*: ↑Incidence foetuses with: - Delayed ossification of sternebrae [10/252 (4%) foetuses (f) or 6/20 (30%) litter (l) Vs. 5/416 (1%) f or 5/34 (15%) l] - Skull foramen [6/252 (2%) f or 6/20 (30%) l Vs. 5/416 (1%) f or 5/34 (15%) l] - Skull bone island [7/252 (3%) f or 6/20 (30%) l Vs. 5/416 (1%) f or 4/34 (12%) l] -**Maternal LOAEL**: 700 mg/kg bw/ day -**Maternal NOAEL**: 300 mg/kg bw/ day **Critical effect at the LOAEL**: ↓ bw gain and ↓ liver weight.	John *et al.* (1978) (CA) B.6.6.2/02

<table>
<tr><th>Method
Guideline.
Deviations if any/Acceptability
Species, strain
Sex
No/group</th><th>Test substance.
Route of expousure
Dose levels, duration of exposure</th><th>Results
- NOAEL/LOAEL (for sexual function and fertility, parents)
- target tissue/organ
- critical effects at the LOAEL

[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)]</th><th>Reference</th></tr>
<tr><td></td><td></td><td>-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></td></tr>
<tr><td>Developmental toxicity, range-finding study, rabbit
OECD 414.
Deviations: Lower than required number of females. Mortality higher than 10%. Necropsy not performed on the day before expected parturition. No examination of foetuses.
Supportive only.
NZW Rabbit.
Females.
7 / Dose group.</td><td>OPP (purity 99.77%)
Oral gavage
0, 250, 500, 750 mg/ kg bw/day from day 7 to 19 of gestation.

Range-finding: study with non-pregnant rabbits in which females dosed with 500 to 1000 mg/kg OPP showed reduced the bodyweight and food consumption .</td><td><u>Maternal toxicity</u>
Mortality: A total of 9 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.
Clinical signs:
<table>
<tr><th rowspan="2">Clinical sign</th><th colspan="4">Dosage (mg/kg bw/day)</th></tr>
<tr><th>0</th><th>250</th><th>500</th><th>750</th></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, weak</td><td>0</td><td>1</td><td>0</td><td>0</td></tr>
</table>
<u>750 mg/kg bw/day</u>:
▪ ↓ bw [(GD 13 (20%)].
▪ ↓ bw gain [(GD 7-10 (302%) and GD 10-13 (1216%)].
▪ 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.
▪ Histopathology:
<table>
<tr><th rowspan="2">Parameter</th><th colspan="4">Dosage (mg/kg bw/day)</th></tr>
<tr><th>0</th><th>250</th><th>500</th><th>750</th></tr>
<tr><td>No. examined</td><td>7</td><td>7</td><td>7</td><td>7</td></tr>
<tr><td>Kidney</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Autolysis</td><td>0</td><td>1</td><td>2</td><td>5</td></tr>
<tr><td>Degeneration tubule(s), bilateral, focal, slight</td><td>0</td><td>2</td><td>3</td><td>0</td></tr>
<tr><td>Degeneration tubule(s), bilateral, multifocal, moderate</td><td>0</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>Degeneration tubule(s), bilateral, diffuse, moderate</td><td>0</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Inflammation, bilateral, focal, slight</td><td>0</td><td>2</td><td>4</td><td>0</td></tr>
<tr><td>Inflammation, bilateral, diffuse, moderate</td><td>0</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Liver</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Autolysis</td><td>0</td><td>1</td><td>2</td><td>5</td></tr>
<tr><td>Stomach</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Erosion (s), mucosa, focal, slight</td><td>0</td><td>0</td><td>0</td><td>3</td></tr>
<tr><td>Pigment-hematogenous-increased, mucosa</td><td>0</td><td>0</td><td>2</td><td>3</td></tr>
</table>
<u>500 mg/kg bw/day</u>:</td><td>Zablotny et al. (1991b) (CA) B.6.6.2/03</td></tr>
</table>

<table>
<tr><th>Method
Guideline.
Deviations if any/Acceptability
Species, strain
Sex
No/group</th><th>Test substance.
Route of expousure
Dose levels, duration of exposure</th><th>Results
- NOAEL/LOAEL (for sexual function and fertility, parents)
- target tissue/organ
- critical effects at the LOAEL

[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)]</th><th>Reference</th></tr>
<tr><td></td><td></td><td>▪ ↓ bw gain [GD 7-10 (101%)].
▪ ↑ kidney abs./rel. wt (15%, ns/34%)
▪ Gross pathology: Pale kidneys.

<u>250 mg/kg bw/day</u>:
▪ ↑ kidney rel. wt (16%, ns)

<u>Reproductive parameters :</u>
No statistically significant differences
<table><tr><th>Dose level (mg/kg bw/day)</th><th>0</th><th>250</th><th>500</th><th>750</th></tr><tr><td>Number bred</td><td>7</td><td>7</td><td>7</td><td>7</td></tr><tr><td>% Pregnant</td><td>100 (7/7)</td><td>100 (7/7)</td><td>100 (7/7)</td><td>85.7 (6/7)</td></tr><tr><td>Number of deaths</td><td>0</td><td>1</td><td>2</td><td>6</td></tr><tr><td>Number moribund</td><td>0</td><td>0</td><td>0</td><td>0</td></tr><tr><td>Pregnancies detected by stain</td><td>0</td><td>0</td><td>0</td><td>0/1</td></tr><tr><td>Number of litters totally resorbed</td><td>0</td><td>0</td><td>0</td><td>1</td></tr><tr><td>Number of viable litters</td><td>7</td><td>6</td><td>5</td><td>0</td></tr><tr><td>Number of corpora lutea/dam</td><td>9.7±3.4</td><td>12.2±2.8</td><td>9.8±2.9</td><td>N.D.</td></tr><tr><td>Number of implantations/dam</td><td>5.7±2.4</td><td>7.5±2.2</td><td>6.0±2.0</td><td>2.0±0.0</td></tr><tr><td>% Preimplantation loss</td><td>40.1±22.5</td><td>35.5±27.4</td><td>37.7±18.8</td><td>N.D.</td></tr><tr><td>Foetuses/litter</td><td>5.3±2.4</td><td>6.3±1.5</td><td>5.2±1.6</td><td>0</td></tr><tr><td>Number of resorptions/litter</td><td>0.4±0.5</td><td>1.2±1.0</td><td>0.8±0.8</td><td>2.0±0.0</td></tr><tr><td>% Implantations resorbed</td><td>7.5 (3/40)</td><td>15.6 (7/45)</td><td>13.3 (4/30)</td><td>100 (2/2)</td></tr><tr><td>% Litter with resorptions</td><td>42.9 (3/7)</td><td>83.3 (5/6)</td><td>60 (3/5)</td><td>100 (1/1)</td></tr><tr><td>Resorptions/litters with resorptions</td><td>1.0 (3/3)</td><td>1.4 (7/5)</td><td>1.3 (4/3)</td><td>2.0 (2/1)</td></tr></table>
-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.
Critical effect at the LOAEL: -</td><td></td></tr>
<tr><td>Developmental toxicity, rabbit
OECD 414.
Deviations: Treatment period ended too soon. Food consumption was not recorded. Mortality was higher than 10%.
Accepted.
NZW Rabbit.
Females.
16 to 24 / Dose group.</td><td>OPP (purity 99.77%)
Oral gavage
0, 25, 100, 250 mg/ kg bw/day from day 7 to 19 of gestation (on day 0 gestation starts and on day 28 surviving animals were sacrificed)</td><td><u>Maternal toxicity</u>
Mortality:
• 1 control ♀ died on day 16 due to umbilical herniation and volvulus of the jejunum. another control ♀ was terminated on day 24 after spontaneous abortion.
• 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.
• 1 ♀ (100 mg/kg bw/day) died on day 14 after inadvertent deposition of the test material into the lungs caused by gavage error.
• 5 ♀ (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 hemorrhage of the gastric mucosa, haemolysed blood within the intestinal tract and decreased content and increased fluidity of ingesta),</td><td>Zablotny et al. (1991c)
(CA)
B.6.6.2/04</td></tr>
</table>

Method Guideline. Deviations if any/Acceptability Species, strain Sex No/group	Test substance. Route of expousure Dose levels, duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL [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)]	Reference
	Range-finding: Doses were based on the previous (*Zablotny et al*., 1991b)	while another ♀ was terminated on day 21 after spontaneous abortion (ulceration and hemorrhage of gastric mucosa, plus evidence suggesting renal toxicity were found). *Clinical signs:* **Dose level (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 toe nail | 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:** ▪ ↓ bw [(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 ingesta. ▪ *Histopathology of the kidney:* **Dose (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 | 3 Inflammation, unilateral, focal: - slight | 0 | 0 | 0 | 1 Inflammation, bilateral, focal: - slight | 0 | 0 | 0 | 3 Inflammation, bilateral, multifocal: - slight | 0 | 0 | 0 | 4 Inflammation, pelvis, unilateral, focal: - slight | 0 | 0 | 0 | 1 Inflammation, pelvis, bilateral, focal: - slight | 0 | 0 | 0 | 2 ***Reproductive and litter parameters:*** *No statistically significant differences* **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).	

Method **Guideline.** **Deviations if any/Acceptability** **Species, strain** **Sex** **No/group**	**Test substance.** **Route of expousure** **Dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL** **[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)]**	**Reference**
		100 mg/kg bw/day ▪ ↑ % litters with resorptions (131%; ns; 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. **Critical effect at the LOAEL**: -	
Developmental toxicity, mice No guideline. **Supportive only** JCL-ICR mice . Females. OPP: 20 to 21 / Dose group. SOPP: 20 / Dose group.	OPP: Oral gavage 0, 1450, 1740 and 2100 mg/kg bw/day from day 7 to 15 of gestation both included. SOPP: Oral gavage 0, 100, 200, or 400 mg /kg bw/day from day 7 to 15 of gestation both included. On day 0 gestation starts and on day 18 surviving animals were sacrificed.	**OPP:** **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. ▪ ↓ bw/bwg (no numerical data available). ▪ ↓ in abs./rel heart wt. (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. 33% mortality ▪ ↓ bw/bwg (no numerical data available). ▪ ↓ in abs./rel heart wt. (9%/7%) and ↑ in rel. liver wt. (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 abs./rel. liver wt. (15%, ndr/17%, ndr) **Litter/reproductive data:** **2100 mg/kg bw/day:** ▪ ↓ fœtal bw in ♂/♀ (20%/20%). ▪ ↑ frequency of foetuses with cervical ribs (17% Vs. 0% in controls) ▪ ↓ mean number of ossified left/right phalanges in forelegs (62%/62%) and hinlegs (44%/44%) and posterior lumbar vertebrae (21%) **1740 mg/kg bw/day:** ▪ ↓ early resorptions (89%) ▪ ↓ fœtal 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%)	Ogata *et al*. (1978) (CA) B.6.6.2-05

<table>
<tr><th>Method
Guideline.
Deviations if any/Acceptability
Species, strain
Sex
No/group</th><th>Test substance.
Route of expousure
Dose levels, duration of exposure</th><th>Results
- NOAEL/LOAEL (for sexual function and fertility, parents)
- target tissue/organ
- critical effects at the LOAEL

[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)]</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
▪ ↑ frequency of foetuses with externally visible malformations (6% Vs. 0.67% in controls)
<u>1450 mg/kg bw/day:</u>
▪ ↓ early resorptions (53%)
▪ ↓ fœtal 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)

Table. External malformations
<table>
<tr><th></th><th colspan="4">OPP (mg/kg bw/day)</th></tr>
<tr><td></td><td>0</td><td>1450</td><td>1740</td><td>2100</td></tr>
<tr><td colspan="5">External malformations[a]</td></tr>
<tr><td>No. litters examined</td><td>20</td><td>14</td><td>14</td><td>5</td></tr>
<tr><td>Cleft palate</td><td>1 [1] 5%</td><td>1 [1] 7%</td><td>4[4] 29%</td><td>1[1] 20%</td></tr>
<tr><td>Open eyelids</td><td>1 [1] 5%</td><td>4 [7] 29%</td><td>6 [6] 43%</td><td>1 [1] 20%</td></tr>
<tr><td>Exencephalia</td><td>0</td><td>3 [6] 21%</td><td>0</td><td>0</td></tr>
<tr><td>Frequency of foetuses with externally visible malformations (All types combined)[b]</td><td>0.67±2.05</td><td>6.21±8.03*
↑826%</td><td>6.14±5.96*↑816%</td><td>3.64±4.98</td></tr>
</table>
a) Number of affected litters, with number of affected foetuses in brackets and the percent of litters affected in brackets, as reported by the investigators.
b) * p<0.05

-Maternal LOAEL: 1450 mg/kg bw/day
-Maternal NOAEL: < 1450mg/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.

<u>SOPP</u>
<u>Maternal toxicity:</u>
<u>400 mg/kg bw/day:</u>
▪ ↑ mortality (80% of unscheduled deaths): 1 mouse died on day 11 of pregnancy, 4 on day 12, 2 on day 13, 1 on day 14, 3 on day 15, 2 on day 16, 2 on day 17 and 1 on day 18 (bleeding from the ostium vaginae was found in almost all the mice that died at all dose levels), presumably attributable to abortions).
▪ ↓ bw/bwg (no numerical data available).
▪ ↓ abs. wt. of liver (14%), heart (10%) and spleen (22%).
<u>200 mg/kg bw/day:</u>
▪ ↑ mortality (20% of unscheduled deaths): 2 mice died on day 15 of pregnancy and 1 each on days 14 and 16.
▪ ↓ bw/bwg (no numerical data available).
▪ ↑ rel. lung wt. (14%, ndr)
<u>100 mg/kg bw/day:</u>
▪ ↓ bw/bwg (no numerical data available).
</td><td></td></tr>
</table>

Method **Guideline.** **Deviations if any/Acceptability** **Species, strain** **Sex** **No/group**	**Test substance.** **Route of expousure** **Dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL** **[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)]**	**Reference**
		Litter/reproductive data: **400 mg/kg bw/day:** ▪ ↓ fœtal bw in ♂/♀ (15%/15%). ▪ ↑ frequency of foetuses with cervical ribs (4.1% Vs. 1.2% in controls) ▪ ↓ mean number of ossified left/right phalanges in forelegs (59%/51%) and posterior lumbar vertebrae (24%) **200 mg/kg bw/day:** ▪ ↓ number of implantation site/dam (14%). ▪ ↓ litter size (live foetuses) (21%) ▪ ↓ fœtal bw in ♂/♀ (8%/8%) ▪ ↓ mean number of ossified left/right phalanges in forelegs (26%/29%) and hinlegs (26%, ndr/30%, ndr) **100 mg/kg bw/day:** ▪ ↓ fœtal bw in ♂/♀ (15%/12%). ▪ ↓ mean number of ossified left/right phalanges in forelegs (27%/25%) and hinlegs (33%, ndr/31%, ndr). -**Maternal LOAEL**: 100 mg/kg bw/day -**Maternal NOAEL**: < 100 mg/kg bw/day **Critical effect at the LOAEL**: ↓ bw gains. -**Developmental LOAEL**: 100 mg/kg bw/day. -**Developmental NOAEL**: < 100 mg/kg bw/day. **Critical effect at the LOAEL**: ↓foetal bw and ↑incidence of skeletal variants.	
Developmental toxicity, meta-study No guideline. **Supportive only (reliable)**.	n.a.	n.a	Kwok *et al.* (2013) (CA) B.6.6.2-06

Table 61: Summary table of human data on adverse effects on development

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No data				

Table 62: Summary table of other studies relevant for developmental toxicity

Type of study/data	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No data				

2.6.6.2.1 Short summary and overall relevance of the provided information on adverse effects on development

There are four developmental toxicity studies performed with OPP (two in rabbits and two in rats), and one mouse

study with OPP and SOPP. These seven studies are included in the original DAR (2008), however the SOPP section of the mice developmental study had not been evaluated until now.

Rats:

-In the first rat developmental toxicity study (Kaneda *et al*., 1978, 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 (9 of 11), 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.

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). Nevertheless, the foetus (not the litter) was the experimental unit for the statistical analysis of resorptions and therefore, the increased resorption in OPP-treated dams may be equivocal. Also included in this article was a dominant-lethal study to assess the effects of OPP on sperm in C3H mice. OPP was administered by gavage to male mice (15/dose) at 0 (aqueous gum Arabic), 100 or 500 mg/kg bw/day for 5 days. Ethyl Methyl Sulfonate (EMS) served as the positive control. Mating was initiated immediately after the final treatment and continued for 6 weeks. Males showed slight decreases in body weight at 500 mg/kg bw/day, in addition to a "temporary depression".

Considering these effects, the **parental and developmental NOAEL for this study were both selected to be 150 mg/kg bw/day**.

-In the second rat developmental toxicity study (John *et al*., 1978, B.6.6.2/02), pregnant rats were dosed with 0, 100, 300 and 700 mg/kg bw/day. The dose levels were based on a range-finding study where, sperm-positive dams (5-6 dams/group) were gavaged at 0, 250, 400, 800, 1200 or 2000 mg/kg bw/day during gestation (dosing days not specified) and sacrificed on GD 16. Deaths occurred only at the high dose tested. Dams exposed to 800 or 1200 mg/kg bw/day exhibited gastric irritation, decreased maternal body weight and decreased food consumption. On this basis, the investigators selected 700 mg/kg bw/day as the high dose for the main study. In the main study, 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.

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-l0 days of treatment. After the scheduled sacrifice, decreases in absolute liver weights where observed during necropsy.

There were no effects on foetal developmental parameters and no external or visceral effects were observed. But 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 historical controls (5% foetuses and 28% litters).

Considering these effects, the **parental and developmental NOAEL for this study were both selected to be 300 mg/kg bw/day**.

Additionally a possible statistically significant increase in pre-implantation loss at 700 mg/kg bw/day has been described by Kwok and Silva (2013, B.6.6.2-06/2), who also describe procedural errors when testing for implantation sites and foetal resorptions that may have resulted in resorptions being underestimated (it is possible that some of the instances of pre-implantation loss at 700 mg/kg bw/day might have been instances of early resorption or post-implantation loss). Unfortunately, historical control data from the conducting laboratory are unavailable for further evaluating the biological significance of this finding.

Rabbits:

-In a range-finding developmental toxicity study in rabbits (Zablotny *et al.*, 1991b, 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 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, does showed body weight reduction and marked body weight gain depression. At 500 mg/kg bw/day, one surviving rabbit aborted two foetuses on GD 20 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. The report did not provide data for foetal examinations. Based on these results, the investigators selected 250 mg/kg bw/day as the high dose for the full study.

-In the main developmental study with rabbits (Zablotny *et al*., 1991c, B.6.6.2/04), OPP was administered at doses of 0, 25, 100 and 250 mg/kg bw/day. As in the probe study (B.6.6.2/03), 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; but the authors dismiss this effect claiming:

- It is not statistically significant and within or marginally above the historical controls (see table on caesarean section and litter data).
- The "number of resorptions per litter with resorptions" does not follow a dose-response curve.
- A WOE analysis (Carney E. and Zablotny C., 2006)[6] supported that, in the probe study on rabbits by Zablotny (B.6.6.2-03) and in the studies with rats (B.6.6.2-01 and B.6.6.2-02), there did not seem to be increase in resorptions, at least not in the absence of significant maternal toxicity.

The **maternal NOAEL was 100 mg/kg bw/day, the developmental NOAEL ≥ 250 mg/kg bw/day**

However an alternative interpretation of these study's data has been proposed by Kwok and Silva (2013, B.6.6.2-06) based on the following counter points:

- The increased incidence of litters with resorptions may be related to the blood detected in the pan, the faeces, or urine during cage side observation.
- The statistical analysis employed in this study is not appropriate. With a suitable statistical analysis, the percent of resorptions per litter exhibits a significant dose-related trend and is significantly increased at 100 and 250 mg/kg bw/day (31%, 57%, 77% and 82% for control, 25, 100 and 250 mg/kg bw/day dose groups). Additionally, the percent litters with resorptions actually clearly exceeded the historical control range (11.1-66.7%).
- WOE argument should be reviewed in the light of the newer analysis by Kwok and Silva. (2013, B.6.6.2-06) of OPP developmental toxicity studies.

If Kwok and Silva are indeed correct, based on the increased litter incidence of resorptions at 100 mg/kg bw/day, the developmental NOAEL could be set at 25 mg/kg bw/day, and developmental toxicity would be present at doses at which maternal toxicity is not. However the RMS remains insuficiently convinced of this to adopt such low developmental NOAEL and proposes maintaining a developmental NOAEL ≥ 250 mg/kg bw/day.

Mice:

-The developmental toxicity study in mice (Ogata *et al.*, 1978, B.6.6.2-05) consisted of two studies: one with OPP and a second one with SOPP:

- In the first (OPP) study, 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

[6] Carney E., Zablotny C. (2006) Developmental toxicity endpoint. Response to Department of Pesticide Regulation *Ortho*-Phenylphenol (OPP) and Sodium *Ortho*-Phenylphenol (SOPP) Risk Characterization Document (RCD). Dietary Expoususre Draft. Lanxess Corporation and the Dow Chemical Company. 27-30

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. A **maternal and developmental NOAEL < 1450 mg/kg bw/ day were set for this study** as both maternal and foetal effects occurred at the lowest dose tested.

- In the second (SOPP) study, four groups of mice bearing vaginal plugs (20 animals/dose) were dosed by gavage at 0 (water), 100, 200, or 400 mg/kg bw/day SOPP on GD 7 through 15 and sacrificed on GD 18. Maternal deaths occurred at 200 and 400 mg/kg bw/day (4 and 16 deaths, respectively). The investigators indicated that each of the SOPP-treated groups had inhibition of the maternal body weight gain. Vaginal bleeding was the only clinical sign noted, and it occurred in all animals that died. The investigators attributed the vaginal bleeding to "abortions."
  Foetuses had decreased body weights at all doses. Decreases in the number of implantation sites per litter and live foetuses occurred at 200 mg/kg bw/day and 400 mg/kg bw/day (although not statistically significant), albeit only four litters were available for examination at laparohysterectomy. The numbers of corpora lutea per dam were comparable among the four groups; however the decreases in the numbers of implantation sites per dam at 200 and 400 mg/kg bw/day were consistent with pre-implantation loss. Ossification of phalanges was significantly reduced in all treated groups, but without apparent dose response. External malformations at 100 mg/kg bw/day increase in the overall incidence.
  The **maternal and developmental NOAEL for this study are both bellow 100 mg/kg bw/ day**, based on reduced body weight gains and on foetal body weight and increased incidence of skeletal variants respectively at 100 mg/kg bw/day.

The study investigators concluded that SOPP and OPP were not teratogenic since there was no dose response at the higher doses in either study, the compounds induced no unique malformation, and most affected foetuses treated originated from a single dam.

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 (Eigenberg *et al*. 1990, 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.

- Numbers are reduced when looking at litters affected, indicative of a heritable effect.

- Historical control data from reproduction studies using albino Crl:CD(SD)BR rats showed that dilated renal pelvis in weanling and cull control animals was common.

On the other hand, in the second two-generation study (Eigenberg and Lake, 1995, B.6.6.1-02), neither clinical alternations nor pathology abnormalities were detected in pups.

Therefore, the overall developmental assessment may not be sufficient for dismissing the possible teratogenic effect based on the following considerations (Kwok *et al*., 2013, B.6.6.2-06):

- Inconsistencies appear when both studies (OPP and SOPP) are considered together i.e. comparable doses of SOPP and OPP led to very different mortality rates, equivalent doses of SOPP triggered bigger changes in foetal body-weight than OPP.
- Dose selection was not optimal. The study was conducted in mice, but OPP dose selection was based on a

rat LD_{50} (while SOPP dose selection was based on a mice LD_{50}). The result of this is that the lowest OPP dose used, is over 3 times higher than the highest SOPP dose, making comparisons between the two substances difficult. Moreover, for all 2 LOAELs (maternal and developmental for OPP and SOPP) were selected at the lowest dose, so it is not possible to know which one appeared first.

- There is no reason to expect that, if OPP and (or) SOPP truly were developmental toxicants, they necessarily would induce a type of malformation that does not occur "spontaneously" in foetuses from control animals.
- With respect to the lack of dose response claimed by the study authors, embryo-foetal death at higher doses is known to reduce the number of foetuses at risk for malformation
- Another study by Ogata *et al.* [7] with thiabendazole, showed a low spontaneous cleft palate incidence in mice compared to SOPP. It should be also noted that the control groups in both (OPP and SOPP) studies had a single foetus with cleft palate.

-The developmental toxicity meta-study by Kwok and Silva (2013, B.6.6.2-06) has been discussed in depth when evaluation the rest of the developmental toxicity studies in this section, as the paper is basically a re-evaluation of the developmental and reproductive toxicity studies with OPP and SOPP summarized and assessed in section 2.6.6 of this document. This study has been instrumental in raising the possibility that OPP and SOPP are developmental toxicants and need to be classified as such, and has heavily influenced the RMS assessment of this hazard category. The conclusions of this re-evaluation for each individual study have been sufficiently explained in this section and in section B.6.6.2 in volume 3. The overall conclusion of this metastudy is that there could be a pattern of developmental effects associated with OPP and SOPP treatment across all species examined. Although further studies are needed to elucidate the developmental toxicity of OPP and SOPP, these re-evaluations indicated that foetal effects (e.g., resorption) occurred in the absence of maternal toxicity.

Overall, 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. This question may have to be revisited in light of the new re-evaluation by Kwok and Silva (2013).

Ortho-Phenylphenol classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). Classification regarding developmental toxicity is not included.

Regarding SOPP, there is a single developmental toxicity study in mice is available (Ogata *et al.*, 1978, B.6.6.2-05). This study was published in Japanese, and although an official translation is available, the reporting is quite incomplete. The study is considered to be of limited validity. In it, SOPP caused effects in dams and foetuses at the lowest dose level, so the maternal and developmental NOAEL for SOPP in mice are both bellow 100 mg/kg bw/ day.

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

CLP criteria regarding reproductive toxicity (which includes adverse effects on development) has already been described in section 2.6.6.1.2 of this document.

If Kwok and Silva are indeed correct, based on the increased litter incidence of resorptions at 100 mg/kg bw/day in the main developmental study in rabbit (Zablotny *et al.*, 1991c, B.6.6.2/04), the developmental NOAEL should be set at 25 mg/kg bw/day, and developmental toxicity would be present at doses at which maternal toxicity is not. In that case classification as toxic for development would be guaranteed.

However the RMS is not sufficiently convinced of this conclusion, at least not enough to classify **OPP** as a developmental toxin without further discussion.

[7] Ogata A, Ando H, Kubo Y, Hiraga K. Teratogenicity of thiabendazole in ICR mice. *Food Chem Toxicol*. 1984;22(7):509-520. doi:10.1016/0278-6915(84)90220-5

2.6.6.3 Adverse effects on or via lactation [equivalent to section 10.10.7 of the CLH report template]

Table 63: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL**	**Reference**
No data			

Table 64: Summary table of human data on effects on or via lactation

Type o data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No data				

Table 65: Summary table of other studies relevant for effects on or via lactation

Type of study/data	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Two-generation, rat study	OPP	-At 457 mg/kg, pup bodyweights was statistically decreased at the end of the lactation period, averaging a 12% difference when compared to controls	This happened only at the highest dose tested, at which adult body weights were also affected	Eigenberg (1990) (CA) B.6.6.1-01
Two-generation, rat study	OPP	Mean Lactation Index ± S.E. (No. of live pups on Day 21/No. of live pups on Day 4 post-culling × 100) Dose group [mg/kg bw/day] | 0 | 20 | 100 | 500 F1a | 100.0± 0.00 | 99.4± 0.63 | 100.0± 0.00 | 99.5± 0.48 F1b | 99.0± 0.72 | 96.3± 2.05 | 99.4± 0.63 | 98.9± 0.75 F2a | 97.5± 1.34 | 100.0± 0.00 | 99.4± 0.63 | 99.5± 0.54 F2b | 98.6± 1.39 | 100.0± 0.00 | 99.4± 0.57 | 99.6± 0.45	Lactation indexes were not affected by treatment	Eigenberg & Lake (1995) (CA) B.6.6.1-02
Repeat dose ADME study in lactating goats	OPP	Radiolabelled OPP was administered to goats for 5 days and its distribution in organs/tissues analysed. Over 86% of the radioactivity was eliminated in the excreta for each group within the five-day dosing period. The animal in the low dose eliminated 82.8 % in the urine and 4.32 % in the faeces whereas the high dose animal eliminated 80.3 % in the urine and 10.2 % in the faeces. The total radioactivity residues (TRR) in milk ranged from 0.006 to 0.008 ppm in the low dose animal and 0.0031 to 0.043 ppm for the high dose animal. The entire milk production for each group contained ≤ 0.10 % of the total	OPP does not preferentially distribute in milk, where it is not present in amounts sufficient to cause concern.	Thalacker F. (1997) B.6.1.1-02

<table>
<tr><th>Type of study/data</th><th>Test substance</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td></td><td></td><td>dose, see table below:

Amount of radioactivity in milk at specified times post-dose
<table>
<tr><td rowspan="2">Collection time</td><td colspan="2">Dose: 13.7 mg/kg bw/day</td><td colspan="2">Dose: 53,3 mg/kg bw/day</td></tr>
<tr><td>µg equiv./ g</td><td>% of dose</td><td>µg equiv./ g</td><td>% of dose</td></tr>
<tr><td>Day 1</td><td>0.006</td><td>0.01</td><td>0.031</td><td>0.02</td></tr>
<tr><td>Day 2</td><td>0.008</td><td>0.02</td><td>0.036</td><td>0.02</td></tr>
<tr><td>Day 3</td><td>0.008</td><td>0.02</td><td>0.039</td><td>0.02</td></tr>
<tr><td>Day 4</td><td>0.008</td><td>0.02</td><td>0.034</td><td>0.02</td></tr>
<tr><td>Day 5</td><td>0.007</td><td>0.02</td><td>0.043</td><td>0.02</td></tr>
<tr><td>Total [%]</td><td>N/A</td><td>0.09</td><td>N/A</td><td>0.10</td></tr>
</table></td><td></td><td></td></tr>
</table>

2.6.6.3.1 Short summary and overall relevance of the provided information on effects on or via lactation

The available information on the potential of OPP and SOPP to cause adverse effects on the offspring via lactation is contained in the two 2-generation reproductive studies by Eigenberg (1990, B.6.6.1-01) and Eigenberg & Lake (1995, B.6.6.1-02) and in an ADME study carried out with goats by Thalacker F. (1997, B.6.1.1-02)

In the generational studies there is no clear evidence of adverse effects in the offspring due to transfer of test substance in the milk, or of adverse effect on the quality of the milk. In the ADME study there is no data indicating that OPP is present in in potentially toxic levels in breast milk.

Ortho-Phenylphenol classification and labelling is listed in Annex VI of Regulation (EC) No. 1272/2008 (it was modified for the last time by Commission Directive 2000/32/EC of 19 May 2000). Classification regarding toxic effects on or via lactation is not included.

2.6.6.3.2 Comparison with the CLP criteria regarding effects on or via lactation

There is no evidence in human or animal studies that OPP is absorbed by women and has been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child.

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity

Based on the data available for ***ortho*-phenylphenol (OPP)**, and according to the criteria under Regulation (EC) No. 1272/2008, RMS proposes **no classification** for this active substance in this hazard class.

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.

<u>Two-generation study in rats (B.6.6.1-01)</u>

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.

<u>Two-generation study in rats (B.6.6.1-02)</u>

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%) Lactation	B.6.6.1-02, 1995

- ↑ 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

<u>500 mg/kg bw/day</u>

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.

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

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.)	Kaneda *et al.*, 1978 B.6.6.2/01

	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).	
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)	**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: ○ Delayed ossification of sternebrae [10/252 (4%) foetuses (f) or 6/20 (30%) litter (l) vs. 5/416 (1%) f or 5/34 (15%)] ○ Skull foramen [6/252 (2%) (f) or 6/20 (30%) (l) vs. 5/416 (1%) (f) or 5/34 (15%)] ○ 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	B.6.6.2/02, 1978

Critical effect at the LOAEL: ↑ incidence of skeletal variants and post-implantation loss.

Development al 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

MATERNAL TOXICITY

B.6.6.2/0 3, 1991b

Mortality: 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.

Clinical signs:

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

750 mg/kg bw/day

- ↓ body weight [(GD 13 (20%)]
- ↓ body weight gain [(GD 7-10 (302%) and GD 10-13 (1216%)]
- 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

500 mg/kg bw/day

- ↓ 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.

Development al 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

MATERNAL TOXICITY

B.6.6.2/04, 1991c

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.
- 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).

<u>Clinical signs</u>

	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),	0	0	0	3
bilateral, multifocal: - moderate	0	0	0	1
Inflammation, unilateral, focal: - slight	0	0	0	3
Inflammation, bilateral, focal: - slight	0	0	0	4
Inflammation, bilateral, multifocal: - slight	0	0	0	1
Inflammation, pelvis, unilateral, focal: - slight	0	0	0	2
Inflammation, pelvis, bilateral, focal: - slight				

REPRODUCTIVE AND LITTER PARAMETERS

<u>250 mg/kg bw/day</u>
- ↑ % 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** <u>2100 mg/kg bw/day</u> • ↑ 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%) <u>1740 mg/kg bw/day</u> • ↑ 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) <u>1450 mg/kg bw/day</u> • ↑ 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** <u>2100 mg/kg bw/day</u> • ↓ foetal bw in ♂/♀ (20%/20%) • ↑ frequency of foetuses with cervical ribs (17% vs. 0% in controls) • ↓ mean number of ossified left/right phalanges in forelegs (62%/62%) and hindlegs (44%/44%) and posterior lumbar vertebrae (21%) <u>1740 mg/kg bw/day</u> • ↓ 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) <u>1450 mg/kg bw/day</u> • ↓ 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)	Ogata *et al.,* 1978 B.6.6.2-05

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.**

2.6.7 Summary of neurotoxicity

Ortho-Phenylphenol (OPP) and sodium *ortho*-phenylphenate (SOPP) bear no structural similarity to organophosphates, carbamates or other known inducers of delayed neurotoxicity. Besides, studies in several species did not indicate the occurrence of neurotoxic effects, and the rapid excretion of OPP and SOPP precludes the bioaccumulation of the compound. No further data on neurotoxicity of the active substances is required according to Regulation (EU) 283/2013.

Table 66: Summary table of animal studies on neurotoxicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results: - NOAEL/LOAEL - target tissue/organ -critical effect at LOAEL**	**Reference**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results: - NOAEL/LOAEL - target tissue/organ -critical effect at LOAEL	Reference

2.6.8 Summary of other toxicological studies

2.6.8.1 Toxicity studies of metabolites and impurities

During the Peer Review of *ortho*-phenylphenol by EFSA and Member states, information on the toxicological profile of phenylhydroquinone (PHQ) was requested with the intent to set specific reference values (EFSA, 2008).

A total of seven studies have been submitted to address this point as part of the renewal assessment of *ortho*-phenylphenol. Five of these studies have been previously evaluated at EU level as part of the Annex I inclusion of *ortho*-phenylphenol and two new studies have been submitted (B.6.8.1-05 and B.6.8.1-07). All studies have been evaluated as part of this review.

The metabolites PHQ and PBQ form DNA adducts in HL-60 cells and cause oxidative damage, which is a human promyelocytic cells that has significant myeloperoxidase activity, an enzyme that oxidises hydroquinone into benzoquinone (Horvath *et al*, 1992, B.6.8.1-01; Murata *et al*, 1999, B.6.8.1-06). PBQ but not PHQ induced micronuclei in V79 cells (Lambert and Eastmond, 1994, B.6.8.1-02). OPP forms DNA adducts *in vitro* when activated by liver microsomomes whereas PHQ and PBQ form adducts with guanosine residues without metabolic activation (Ushiyama *et al*, 1992, B.6.8.1-03). The generation of PBQ adducts with DNA has indicated that guanine is the preferred nucleobase for DNA adduction by PBQ (Zhao *et al*, 2002, B.6.8.1-04). PHQ caused mitotic arrest and apoptosis at cytotoxic concentrations (Imai *et al*, 2009, B.6.8.1-05). A QSAR analysis suggests that PHQ possesses similar or greater toxicity than parent OPP (Mostert, 2016, B.6.8.1-07). PHQ may undergo oxidation to PBQ which is suspected to produce cytotoxicity. Furthermore, phenylhydroquinone (PHQ) and phenylbenzoquinone (PBQ) are clastogenic in the presence and absence of metabolic activation (Tayama and Nakagawa, 1991, B.6.4.1.3-06).

The formation of PHQ in mice following subchronic exposure to OPP is > 10 % in urine whereas PHQ detected in rats was ~ 5 % (Bartels *et al*, 1998, B.6.1.1-05). The ADI for OPP has been defined based on a 2-year combined chronic toxicity/carcinogenicity study in rats (Washle and Christenson, 1996, B.6.5-02) in which structural alterations in the urinary bladder were observed in males at 200 mg/kg bw/day. The NOAEL was 39 mg/kg bw/day, dose at which no effects in the bladder were observed. The amount of PHQ metabolite formed in rats is less than 10 % (value obtained from Batels and McNett, 1996, B.6.1.1-06) and therefore, the migration of the reference value from the parent may not be applied. No reference value can be determined for PHQ so the toxicological relevance of this metabolite remains to be determined:

- Gene mutation: This may be covered by the QSAR analysis
- Aneugenicity/Clastogenicity: Data gap
- Repeat dose (extended 28-day or 90-day studies): Data gap

2.6.8.2 Supplementary studies on the active substance

2.6.8.2.1 Summary of mechanistic studies

The mechanistic studies outlined in table 67, have been evaluated in more detail in section 2.6.5.1.

Table 67: Summary table on supplementary studies.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, route of exposure duration of exposure	Observations	Reference

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, route of exposure duration of exposure	Observations	Reference
Subchronic study into bladder effects. No guideline. **Supportive only**. CDF[F-344]/BR rats Males. 20 /group.	OPP 1000, 4000 or 12,500 ppm, in diet *ad libitum, for 13 weeks.*	OPP caused morphological alterations of the urinary bladder epithelium in the highest dose group. NOAEL = 4000 ppm (~224 mg/kg bw/day)	Christenson *et al.* (1996a) (CA) B.6.8.2-02
Subchronic ^{32}P-post labelling study. No guideline. **Supportive only** CDF[F-344]/BR rats Males. 22 /group.	OPP 1000, 4000 or 12,500 ppm, in diet *ad libitum*, for 13 weeks.	Increase of mitotic activity and hyperplasia of the urothelium at dose levels ≥ 8000 ppm. No DNA adducts. NOAEL = 4000 ppm (~285 mg/kg bw/day).	Christenson *et al.* (1996b) (CA) B.6.8.2-03
32-week, dietary, No guideline. **Supportive only** F344 rats Males. 30 to 31 rats /group.	12,500 ppm(OPP) 20,000 ppm (SOPP), with varying amounts of $NaHCO_3$,in diet *ad libitum*, for 104 weeks.	SOPP is carcinogenic in rat urinary bladder, while OPP is not. Morphological changes of the bladder epithelium, correlating with increased urinary pH and Na+ concentration.	Fukushima *et al.* (1989) (CA) B.6.8.2-04
12-week study. No guideline. **Supportive only**. F344 rats. Males. ~30/group.	2.0% SOPP for 64 weeks (experiment 1) 2.0% OPP for 64 weeks (experiment 2) SOPP : 0, 2500, 5000, 10,000, 20,000 ppm, *ad libitum* in diet for 36 weeks (experiment 3).	Under the conditions of this study administration OPP after BBN treatment had no significant tumour-promoting activity whereas SOPP acted as a tumour promoter. At 20,000 ppm: morphological changes of the bladder luminal surface evident by SEM.	Fukushima *et al.* (1985) (CA) B.6.8.2-05
In-vitro metabolism of PHQ No guideline. **Supportive only**.	0.2 mM PHQ incubated with 200 U PGHS.	PHQ can be metabolised in-vitro by PGHS yielding PBQ. Prostaglandins and the metabolism of araquidonic acid may play an important role in the detoxification processes of OPP and their metabolites.	Kolachana *et al.* (1991) (CA) B.6.8.2-06
In-vitro metabolism of PHQ and PBQ. No guideline. **Supportive only**.	0.05-0.5 M solution of PHQ or PBQ.	Autoxidation of PHQ to PBQ is accelerated when pH values increase. The presence of PBQ and O_2 further accelerates this reaction.	Kwok & Eastmond (1997) (CA) B.6.8.2-07
Tumour initiation / promotion. No guideline. **Supportive only**. F344 rats. Males. 30/ group.	20,000 ppm OPP or SOPP, in the diet for 32-weeks.	SOPP acts as a tumour promoter following initiation by BBN. SOPP alone also induced tumour formation and can therefore be considered a weak initiator. OPP had no significant tumour-promoting or -initiating effects.	Fukushima *et al.* (1983) (CA) B.6.8.2-08
Carcinogenicity study No guideline. **Supportive only**. F344/DuCr rats. Males. 31/group.	12,500 ppm (OPP), 20,000 ppm (SOPP), with/without $NaHCO_3$ in the diet for 26 weeks.	Urinary bladder tumorigenesis by OPP is enhanced by $NaHCO_3$. Conversely, the carcinogenic potential of SOPP is reduced by co-administration of an acidifier, NH4Cl, which made it less potent than OPP	Fujii *et al.* (1987) (CA) B.6.8.2-09
Carcinogenicity study No guideline. **Supportive only**. F344 rats Males. 15/group.	20,000 ppm OPP or SOPP, dietary for 32-week.	Reduced urinary osmolality. Increased pH and Na^+ correlate with tumorigenesis.	Fukushima *et al.* (1986) (CA) B.6.8.2-10
Mechanistic, DNA-binding study. No guideline. **Supportive only.**	(Short-term)OPP, SOPP: 2% in diet for 90-day. (Acute)OPP, SOPP: 500	SOPP, but not OPP, caused regenerative hyperplasia of the urinary bladder. OPP-treated rats revealed renal damage. No interactions with DNA could be demonstrated	Reitz *et al.* (1983) (CA)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, route of exposure duration of exposure	Observations	Reference
F344 rats Males. 30 or 8/group (short-term or acute).	mg/kg by gavage for 16 hours.	for either compound.	B.6.8.2-11
Carcinogenicity study. No guideline. **Supportive only.** F344 rats Both sexes. 5 or 6 / group and sex.	OPP: 1.25% with or without $NaHCO_3$ SOPP: 2% with or without NH_4Cl. In the diet for 8 weeks.	Males are more sensitive to OPP than females under alkalinuric conditions with respect to bladder hyperplasia.	Hasegawa *et al.* (1991) (CA) B.6.8.2-12
Carcinogenicity study. No guideline. **Supportive only.** F344 rats Males. 5 / group (agglutination assay). 40 rats and a control group of 20 rats (*in-vivo* carcinogenesis experiment).	OPP, SOPP: 0.1-2.0% dietary for 1-week (agglutination assay) or 50-weeks (*in-vivo* carcinogenesis experiment).	OPP and SOPP caused a dose-dependent increase in agglutinability of bladder epithelial cells by Con A which is an indication for carcinogenic potential. SOPP caused carcinomas or preneoplasic lesions in urinary bladder and also but with lower incidence in renal pelvis of male rats.	Honma *et al.* (1983) (CA) B.6.8.2-13
Mechanistic No guideline. **Supportive only.** F344 rats Males. 4 / group.	OPP, PHQ, PBQ: 700, 1400 mg/kg bw., single oral gavage, with or without inhibition of GSH synthesis.	OPP treatment led to GSH depletion and liver and kidney damage. Inhibition of GSH synthesis aggravated hepatotoxicity of OPP. In addition, an intermediate of OPP (PBQ) induced hepatic and renal damage as well.	Nakagawa & Tayama (1988) (CA) B.6.8.2-14
In-vitro cytotoxicity test in primary male F344 rat hepatocytes.	OPP, PHQ: 0–1 mM	OPP cytotoxicity is enhanced by monooxygenase inhibition and GSH depletion. PHQ-induced cell death can be inhibited by sulfhydryl compounds.	Nakagawa *et al.* (1992) (CA) B.6.8.2-15
In-vitro metabolism of OPP and its metabolites.	OPP: 1-100 µM	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 (redox cycling).	Roy D.(1990) (CA) B.6.8.2-16
In-vivo assay of DNA synthesis in bladder. No guideline. **Supportive only.** F344 rats Males. 20 / group.	OPP, SOPP: 2% in diet; for 4–24 weeks.	OPP and SOPP cause a proliferative response in renal pelvis and papilla when given at a dietary level of 2%.	Shibata *et al.* (1989) (CA) B.6.8.2-17
In-vitro and *in-vivo* GSH conjugation. No guideline. **Supportive only.** F344 rats Males.	*In-vitro* study: 79 µg/mL *In-vivo* study: 1000 mg/kg, single oral dose.	PHQ-GSH is excreted via the bile after OPP administration to rats. *In-vitro*, PHQ-GSH can be formed non-enzymatically from PBQ and GSH or enzymatically from OPP and GSH.	Nakagawa & Tayama (1989) (CA) B.6.8.2-18
In-vitro interaction with PGHS. No guideline. **Supportive only.**	OPP, PHQ, PBQ: 100 µM	OPP and PHQ stimulate cyclooxygenase activity and are oxidised by PGHS. OPP, PHQ and PBQ inhibit PGHS at higher concentrations.	Freyberger (1994) (CA) B.6.8.2-19
Ten-week feeding study in rats. No guideline. **Supportive only.** F344 rats Males. 10 to 13 / group.	OPP: 1.25% in diet SOPP: 2.0% in diet for 10 weeks.	OPP and SOPP caused urothelial hyperplasia in rats as evident by histology and increased cell proliferation.	St. John *et al.* (2001) (CA) B.6.8.2-20
In-vitro and *in-vivo* macro-molecular binding assay. No guideline. **Supportive**	^{14}C-OPP: 1 µCi *In-vivo*: OPP, SOPP: 50-500 mg/kg, oral	A non-linear increase in macromolecular binding of OPP and SOPP was observed *in-vivo* and *in-vitro*. This may be caused by the	Reitz *et al.* (1984)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, route of exposure duration of exposure	Observations	Reference
only. F344 rats Males. 4 / group.	gavage, 16-18 h.	saturation of detoxification pathways.	(CA) B.6.8.2-21
In-vivo assay of DNA and protein adducts in rats. No guideline. **Supportive only.** F344 rats Males.	*In-vivo:* 0, 15, 50, 125, 250, 500, 1000 mg/kg bw OPP, single oral gavage.	OPP or its metabolites form protein, but not DNA, adducts in urinary bladder tissue.	Kwok *et al.* (1999) (CA) B.6.8.2-22
Enzyme induction study in mouse liver. No guideline. **Supportive only.** Males B6C3F1 mice. 3 dose/time point.	500, 1000 mg/kg bw/day OPP in the diet for 7 or 14 days.	Among the nuclear receptors AhR, CAR, PXR, and PPARα, only PPARα mediated gene expression was elevated following OPP exposure	Geter *et al.* (2009) (CA) B.6.8.2-23
In-vitro PXR transactivation assay. No guideline. **Supportive only.**	0.1 - 10 µM OPP	OPP leads to transactivation of the human PXR, but not of the murine PXR.	Kojima *et al.* (2011) (CA) B.6.8.2/24

2.6.8.2.2 Summary of studies on immunotoxicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, route of exposure duration of exposure	Observations	Reference
Immuno–toxicity Study. No guideline. **Supportive only**. B6C3F1 mice Females. 7 to 10 /group.	OPP Gavage 10, 200, and 2000 mg/kg bw/day, oral gavage, for 10 days over a 2-week period.	OPP did not suppress the immune function of mice	Luster *et al.* (1981) (CA) B.6.8.2-01

-In this immuno-toxicity study in mice (Luster *et al.*, 1981, B.6.8.2-01). The effects of OPP on immunological functions and host susceptibility to infectious agents were examined against a positive control. OPP was administered at 10, 200, and 2000 mg/kg bw/day for 10 days.

At sacrifice, blood samples were taken via cardiac puncture and body, liver, spleen, kidney and thymus weights were recorded. Samples of the brain, lung, liver, kidney, spleen, thymus, salivary gland, adrenal, vagina, bone marrow (sternum), and uterus were fixed and processed for histological examination. At 2000 mg/kg bw/day statistically significant increased relative spleen and thymus weights. Erythrocyte counts were significantly elevated in the two highest OPP-dose groups. **OPP had no other effect on the immune-related parameters measured**, while the positive control group treated with Cyclophosphamide strongly impaired immune function in all of the measured parameters.

Guidelines for: chronic/subchronic, reproductive toxicity, ADME, and other studies; include a range of immune parameters that are often sufficient to identify if a chemical has immunotoxic potential.

The following studies were also reviewed for evidence of immunotoxicological potential of OPP/SOPP:

-Repeat-dose studies in rats, mice, dogs and rabbits were reviewed for treatment-related changes in a variety of indicators of potential immunotoxicity, including: haematology (white blood cells, platelets), clinical chemistry (albumin, globulin and albumin/globulin ratio), macroscopic findings (lymph nodes, thymus, and spleen), organ weights (spleen and thymus), and histopathology findings (lymph nodes, spleen, thymus).

-ADME studies were reviewed for evidence that OPP/SOPP is/are preferentially distributed into immune organs such as: spleen, lymph nodes and thymus.

-Reproductive and developmental toxicity studies were reviewed in search for any potential impact of OPP/SOPP exposure on the developing immune system.

-In general, all toxicological tests carried out with OPP/SOPP and summarized in Volume 3 section B.6 of this assessment report, were also reviewed for instances of diseases that have environmental risk factors and are associated with immune dysfunction (mainly autoimmune, infectious or inflammatory diseases such as leukaemia, asthma, sepsis, lupus, diabetes, etc.), as well as for instances the above mentioned indicators of potential immunotoxicity.

Based on the available apical toxicology data, no treatment related changes in the immunotoxicological sensitive parameters were observed. In addition, OPP and SOPP do not belong to a class of chemicals (e.g., the organotins, heavy metals, or halogenated aromatic hydrocarbons) that would be expected to be immunotoxic. Within the scope of this brief analysis, **it can be concluded that OPP is devoid of immunotoxicological potential**.

2.6.9 Summary of medical data and information

Medical data for *ortho*-phenylphenol (OPP) include some epidemiological studies where few cases of contact allergy to OPP were reported. These data can be find in section 2.6.2.7 of this volume, and, in more detail, in chapter B.6.9 of volume 3 (CA), section B6.

No more effects relevant for classification were included in this section.

No specific medical data was provided for sodium *ortho*-phenylphenate (SOPP).

2.6.10 Toxicological end points for risk assessment (reference values)

Table 68: Overview of relevant studies for derivation of reference values for risk assessment

Species	Study (method/type, route of exposure, length)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
F344/DuCrj rats. Both sexes.	Subchronic. 13-week,dietary.	OPP	↑ relative bladder weights (♂) with onset of abnormal urothelial growth.	761 mg/kg bw/day	1669 mg/kg bw/day	Zempel & Szabo (1993) B.6.3.3-01
Fischer 344 rats. Both sexes. .	Subchronic. 21-day, dermal.	*OPP*	-	Systemic: 1000 mg/kg bw/day.	Systemic: >1000 mg/kg bw/day.	Iguchi *et al.* (1984) B.6.3.2-02
			Local irritation (♂,♀).	Local/dermal: <100 mg/kg bw/day	Local/dermal: 100 mg/kg bw/day	
Fischer 344rats. Both sexes.	Combined Chronic Toxicity/carcinogenicity. 2-years, dietary.	*OPP*	Structural alterations in the urinary bladder (♂).	Systemic: 800 ppm (39 mg/kg).	Systemic: 4000ppm (200 mg/kg).	Wahle & Christenson (1996) B.6.5-02
			Neoplasms (malignant and benign) in the urinary bladder (♂).	Neoplastic: 4000ppm (200 mg/kg).	Neoplastic: 8000 ppm (402 mg/kg).	
B6C3F1 mice. Both sexes.	Combined Chronic Toxicity/carcinogenicity. 2-years, dietary.	*OPP*	↑ liver weights, changes in hepatocytes and tubule morphology (♂,♀), ↓ bw/bwg (♀).	Systemic: <250 mg/kg.	Systemic: 250 mg/kg	Quast & McGuirk (1995) B.6.5-04
			↑ incidence of hepatocellular adenoma (♂).	-Neoplastic : 250 mg/kg.	Neoplastic: 500 mg/kg.	

2.6.10.1 Toxicological end point for assessment of risk following long-term dietary exposure – ADI (acceptable daily intake)

OPP:

The acceptable daily intake (ADI) for humans is normally derived from the NO(A)EL in the most susceptible species in long-term toxicity studies, and an appropriate safety factor. The most sensitive specie was the rat. The NO(A)EL (derived from the database for chronic studies in rat) which best meets the criteria comes from a 2-year combined chronic toxicity/carcinogenicity study in rats (Washle, B.S. and Christenson Christenson, W.R., 1996, B.6.5-02), in which structural alterations in the urinary bladder were observed in males at 200 mg/kg bw/day. As discussed in previous experts meeting a safety factor of 100 would be appropriate, thus the ADI is calculated as follows:

ADI = (39mg/kg bw/day)/ 100 ≈ 0.40* mg/kg bw/day

**This value was published in the EFSA conclusions (2008) 217, 1-67*

2.6.10.2 Toxicological end point for assessment of risk following acute dietary exposure - ARfD (acute reference dose)

OPP:

As published in the EFSA conclusion (2008) 217, 1-67, no ARfD was allocated for ***ortho*-phenylphenol (OPP)** during the previous assessment.

Ortho-Phenylphenol is corrosive to skin (Skin Corr. 1; H314), causes serious eye damage (Eye Dam.1) and is suspected to cause cancer (Carc. 2; H351). However, the test substance showed low acute oral, dermal or inhalation toxicity, developmental studies showed no toxicity effects, no neurotoxic effects were observed in studies performed in several species (and therefore, no specific neurotoxic studies are considered required), and the critical observed effect in short-term toxicity was the abnormal growth of bladder *urothelium* in rats (which is not expected to be produced after an acute exposition (one or few doses) to the substance).

Therefore, based on the available data provided for the renewal assessment, and according to the Guidance for the setting of an Acute Reference Dose (7199/VI/99 -5 July 2001), acute effects observed with *ortho*-phenylphenol are not likely to be relevant for the establishment of an ARfD.

No ARfD has been allocated since it is not considered necessary for *ortho*-phenylphenol (OPP).

2.6.10.3 Toxicological end point for assessment of occupational, bystander and residents risks – AOEL (acceptable operator exposure level)

OPP:

RMS also considers that the AOEL value set in the previous assessment (0.4 mg/kg bw/day) should be maintained.

The AOEL is defined on the basis of short-term toxicity studies in the most sensitive specie and with the application of an appropriate safety factor. In this case due the conditions of use of OPP a long-term AOEL was considered more appropriate. The most sensitive specie was the rat. The NO(A)EL (derived from the database for chronic studies in rat) which best meets the criteria comes from a 2-year combined chronic toxicity/carcinogenicity study in rats (Washle, B.S. and Christenson Christenson, W.R., 1996, B.6.5-02), in which structural alterations in the urinary bladder were observed in males at 200 mg/kg bw/day. The AOEL is calculated as follows:

AOEL= (39mg/kg bw/day)/ 100 ≈ 0.40* mg/kg bw/day

**This value was published in the EFSA conclusion (2008) 217, 1-67.*

2.6.10.4 Toxicological end point for assessment of occupational, bystander and residents risks – AAOEL (acute acceptable operator exposure level)

OPP:

Based on the low acute effects observed with ***ortho*-phenylphenol** (OPP) and following the Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products (SANTE-10832-2015 rev. 1.7 of 24 January 2017), since an ARfD value has not been deemed required for *ortho*-phenylphenol, no AAOEL assessment is necessary for this active substance.

2.6.11 Summary of product exposure and risk assessment

The operator exposure to 2-phenylphenol from the proposed use of AGF/1-04 indicate that the risk to the operator is acceptable without PPE. Therefore, it can be concluded that the risk of operator exposure during the drenching process is very low. Volume 3, Annex B.6.4.1.

The bystander/resident exposure to *2*-phenylphenol from the AGF/1-04 during the treatment is not relevant. Treatment of citrus is performed automatically and no bystander or residents are to be expected walking around the drenching device, which is moreover a closed system. Volume 3, Annex B.6.4.2.

The results of the worker exposure indicate that the risk to residues of 2-phenylphenol is acceptable with PPE (chemical protective gloves, 99% protection). So, there is not unacceptable risk for the worker when handling treated fruit with AGF/1-04, with the use of chemical protective gloves. Volume 3, Annex B.6.4.3

In conclusion : The operator, bystander/resident and worker risk assessment demonstrates acceptable risk to 2-phenylphenol for the proposed use of AGF/1-04 for operators and workers.

However, AGF/1-04 with regards to human health is classified as Carc. 2 (H351), and based on this classification and the requirement for chemical protective gloves for workers, the following PPE are recommended:

- Operator: Work wear (arms, body and legs covered) and chemical protective gloves when handling the concentrate, or handling contaminated surfaces.

  NOTE: according EFSA Guidance, 2014, the penetration factor of the “workwear” is 10 %, equivalent to a type 6 chemical protective coverall (or the correspondent coverall according UNE-EN ISO 27065:2017)

- Worker: Work wear (arms, body and legs covered) and chemical protective gloves when handling treated fruits.

2.7 RESIDUE

2.7.1 Summary of storage stability of residues

Results of storage stability studies in plants show that residues of OPP are stable in orange whole fruits and peel under frozen conditions up to 212 days (7 months), orange pulp up to 206 days (6.8 months), juice and dry pomace up to 60 days (2 months), and citrus oil up to 100 days (3.3 months).

Residues of the metabolite PHQ are stable in orange whole fruits and pulp under frozen conditions up to 211 days (7 months), and juice and dry pomace up to 60 days (2 months). However, PHQ is not stable under frozen conditions in orange peel and oil.

Table 2.7.1-1: Summary of stability data achieved for OPP at ≤ - 18°C

Matrix	Characteristics of the matrix	Acceptable Maximum Storage duration	Reference
EU Reviewed Data			
Orange, whole fruit	High acid content	212 days	Mewis, A. (2012), EU agreed (Spain, 2013)
Orange, peel	High acid content	212 days	
Orange, pulp	High acid content	206 days	
Not EU Reviewed			
Orange, whole fruit	High acid content	60 days	Driss, F. (2019) New data
Orange, juice	High acid content	60 days	
Orange, dry pomace	High acid content	60 days	
Orange, oil	High oil content	100 days	

Table 2.7.1-2: Summary of stability data achieved for PHQ at ≤ - 18°C

Matrix	Characteristics of the matrix	Acceptable Maximum Storage duration	Reference
EU Reviewed Data			
Orange, whole fruit	High acid content	211 days	Mewis, A. (2012), EU agreed (Spain, 2013)
Orange, peel	High acid content	Not stable	
Orange, pulp	High acid content	211 days	
Not EU Reviewed			
Orange, whole fruit	High acid content	60 days	Driss, F. (2019) New data
Orange, juice	High acid content	60 days	
Orange, dry pomace	High acid content	60 days	
Orange, oil	High oil content	Not stable	

No any storage stability study for animal commodities has been submitted in order to support the intended uses. However, since not any animal feeding studies were required according to the intended uses, those storage stability studies are not considered necessary.

2.7.2 Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

The metabolism of [ring-UL-14C] SOPP was investigated in stored oranges and stored pears after treatment by dipping in a dosing solution.

The translocation and metabolism of [*ring*-UL-^{14}C]sodium ortho-phenylphenate ([^{14}C]SOPP) was investigated in oranges after treatment by dipping in a dosing solution at 0.1% or 0.5% SOPP, corresponding to 0.88 g OPP/L or 440 g OPP/L. Oranges treated at 0.1% were sampled and analysed on 9 occasions, between 2h to 12 weeks after treatment/storage, oranges treated at 0.5% were sampled after 13 weeks of storage.
The lower application rate somewhat exceeded the GAP rate (+32%) (60 g OPP/hL). Since metabolism in oranges treated at 0.1% and 0.5% showed the same metabolic pattern, this deviation is not considered significant.
After SOPP was applied on oranges that were then placed in cold storage, it migrated from the surface of the fruit into the peel, but further translocation leading to residues in pulp and juice was limited. The parent compound was relatively stable under the test conditions used. Only a small amount was metabolised to PHQ and 2-methoxybiphenyl (2-MBP) (phase-I metabolites). Small amounts of OPP and PHQ were subsequently conjugated with glucose or other endogenous molecules to form phase-II metabolites.
Free OPP and its glucose conjugate and/or other conjugates of OPP were the major metabolites identified in orange peel (84.51%). The other metabolites identified in peel were PHQ and its conjugates (6.88%). In pulp and juice, OPP was the only metabolite identified that consisted of 0.1% of the TRR of each matrix. Rinse contained OPP (1.33%) and 2-MBP (0.27%). In each orange matrix, there were also some minor unknowns, however, none of them exceeded 3.16% of TRR, which was found in rinse.
Free OPP, PHQ and their respective conjugates can therefore be defined as the relevant residues of OPP in oranges.

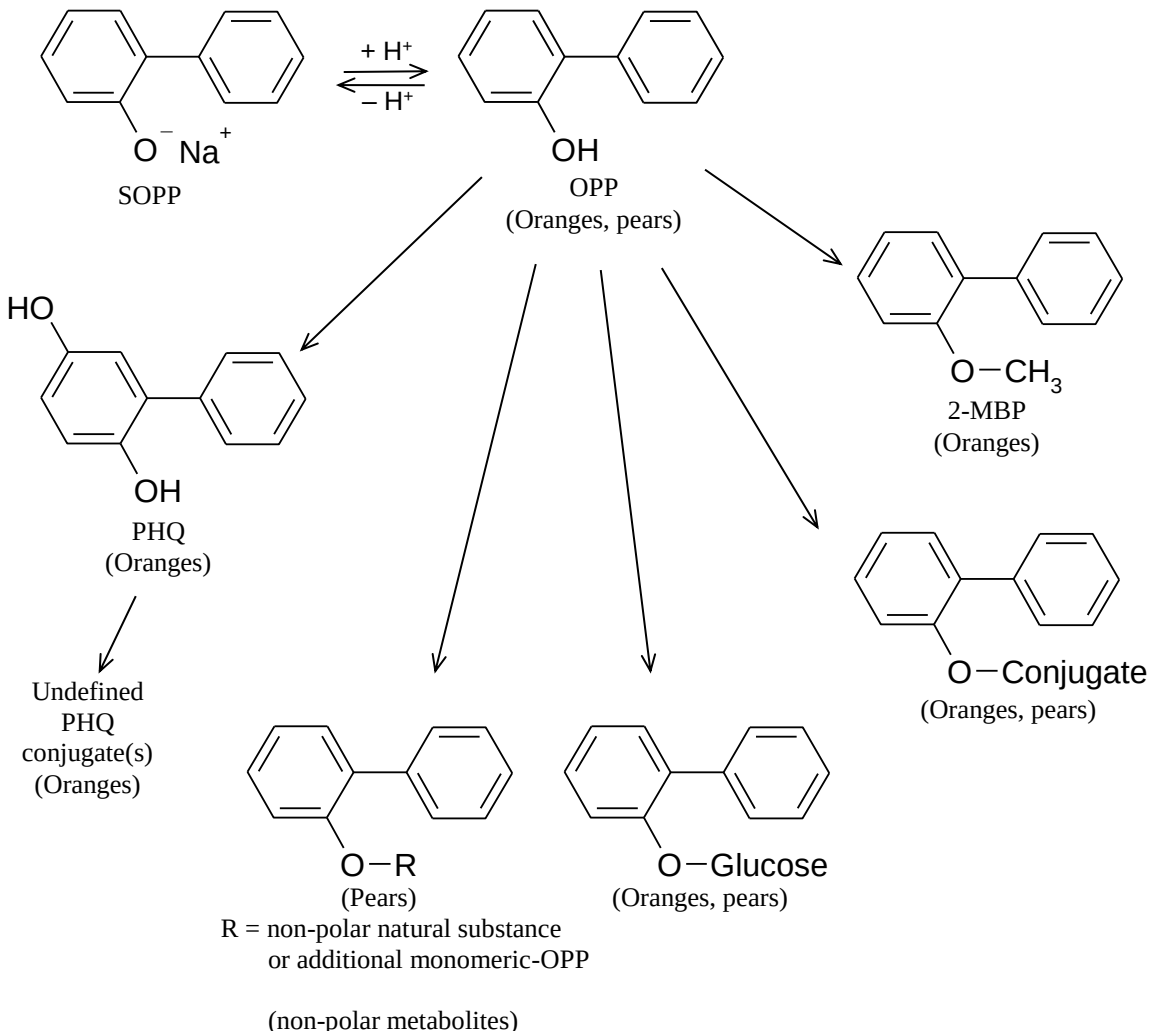
The metabolim study in citrus fruits was well performed and reported. The majority of the radioactivity was detected in fruit rinses and peel, only small amounts were found in pulp and juice. OPP was the major substance identified. A small amount of OPP was metabolised to PHQ and 2-MBP. Small quantities of OPP and PHQ were conjugated with glucose or other endogenous molecules. Summarizing, free OPP, PHQ and their respective conjugates can be defined as the relevant residues of OPP in oranges.

According to EFSA Scientific Report (2008) 217, 1-67, the PRAPeR 60 (round 12) meeting discussed whether the study was representative of the commercial practice. The study was carried out for a period of 12 weeks only whereas according to information of the Rapporteur Member State oranges are stored for up to six months after post harvest treatment. It was concluded that due to the fact that the fruits were stored at a higher temperature during the first 4 weeks the metabolism was increased during this time and the metabolism observed at the end of the study might represent a longer commercial storage period. Furthermore, it was discussed whether unidentified radioactive residues in rinse and peel of the treated fruits were of concern. On the basis of additional information submitted by the notifier on the characterisation of the radioactive residues it was decided that identification/characterisation of metabolites was sufficient.

After dipping in a 4% solution for 3 minutes (representing approximately 12 times the dose rate of the notified citrus fruits cGAP), treated pears were kept in cold storage at approximately -1 to 4 °C for 28 weeks. Samples of fruit were taken for analysis 2 hours, 2 days and 1, 2, 4, 6, 8, 12, 16, 20 and 28 weeks after the application. The amount of total radioactive residues found in the whole fruits was 22 mg/kg two hours after the treatment, increased to 57 mg/kg by day two and afterwards remained relatively constant throughout the study at approximately 40 mg/kg. Penetration of residues from the surface of the fruits into the peel and the pulp was observed. TRR in the peel and the pulp increased to approximately 70% and 30% respectively within 28 weeks of storage. Metabolites were analysed in samples stored for 28 weeks. The main residues found in extracts of the different fractions of the fruits were 2-phenylphenol (parent compound) (6% of TRR) and its conjugates (74% of TRR). Rinse and peel contained also the unidentified metabolite C and further polar and non-polar unidentified compounds. Post extraction solids of peel and pulp were further characterised by hydrolysis steps which released conjugates of 2-phenylphenol. The PRAPeR 60 meeting discussed the validity of the study. The notifier could not provide a conclusive explanation for the low TRR found in samples 2 hours after treatment. The PRAPeR 60 meeting suggested that it could be explained by loss during handling of the samples. The results from days 2 to 28 weeks were regarded as conclusive. The PRAPeR 60 meeting concluded that the unidentified metabolite C was expected at very low concentrations after application of 2-phenylphenol at the notified dose rate and therefore further efforts to identify the residues were not required.

The proposed metabolic pathway of orthophenylphenol in stored pears and oranges is shown in the Figure 2.7.2-2.

Figure 2.7.2-2 Proposed metabolic pathways of OPP in stored oranges and pears



Free OPP, PHQ and their respective conjugates can be defined as the relevant residues of OPP in oranges. On the other hand, free OPP and its conjugates could be defined as the relevant residue of OPP in pears. However, to kept consistency on both metabolic pathways (pears as well as citrus), RMS proposes to define OPP and PHQ as well as their conjugates as the relevant residues for OPP in pome and citrus fruits.

According to the dietary burden calculation (Animal model, 2017 ; see B.7.4) the trigger level of 0.004 mg/kg bw/day is exceeded for cattle (dairy and meat) and breeding swine.

However it should be reminded that the intended uses are post-harvest uses for citrus fruits. Only citrus dried pulp can be used for feed livestock, and it constitutes of the combination of the remaining pulp and peel after drying of the by-product of the juicing process. It should be emphasized that in the common industrial practice, the fruits used for processing into juice are not treated with OPP, and therefore OPP should not be present in citrus processing products intended for animal feed. Even so, a ruminant metabolim study is available. The available study was well performed and reported. Metabolism of OPP in lactating ruminants (goats) was determined. The most abundant residues were found in urine (80.3% to 82.8% of the total dose administered). The majority of residues were excreted within 24 hours of dose administration. Residues were detected in milk (0.009% to 0.1% of total dose administered). Residues were detected in liver (0.01% of total dose administered) and in kidneys (<0.005% of total dose administered). Because of the low concentrations of radioactive residues in the tissues, no metabolites were identified and no metabolic pathway of [^{14}C]OPP in lactating goats can be proposed. Some deviations from the current test guideline OECD 503 were indicated, but taking into account the obtained results at

two exxagerated doses, these deviations do not affect the integrity and validity of the study. According to the results of the metabolism study, no residues are expected in animal commodities at the calculated dietary burden.

The only relevant feed commodity for the intended uses of OPP is the citrus dried pulp. Citrus dried pulp is a feed item only relevant to cattle and breeding swine. Therefore, metabolism studies on poultry are not required.

According to the dietary burden calculation (Animal model, 2017 ; see B.7.4) the trigger level of 0.004 mg/kg bw/day is exceeded for cattle (dairy and meat) and breeding swine. Although metabolites were not identified in lactating goats, it does not become apparent that metabolic pathways differ significantly in the rat as compared to ruminants. Hence it is safely assumed that OPP metabolism for pigs follows a similar pattern as for ruminants, and most of the residues will be excreted via urine and faeces within 24 hours of dose administration. According to Commission Regulation (EU) No. 283/2013, metabolism studies on pigs are necessary where it becomes apparent that metabolic pathways differ significantly in the rat as compared to ruminants. Since it does not seem the case, metabolism studies in pigs are not considered to be necessary according to the intended uses.

No studies on metabolism in fish were included in the Applicant´s submission in support of the first inclusion of OPP in Annex I of Directive 91/414/EEC since this was not a data requirement at the time. Currently, the fish metabolism is a data requirement. However according to SANCO 11187/2013, citrus fruit and their processing products are not considered as commodities commonly used for the formulation of aquaculture diets (see Annex 2. Feedingstuffs table). Therefore, the use of OPP according to the intended uses is not foreseen to affect fishes feeding.

2.7.3 Definition of the residue

Plant residue definitions:

Regarding the metabolism studies in oranges and pears, free OPP, PHQ and their respective conjugates could be defined as the relevant residues of OPP in fruits. Applicant has proposed a plant monitoring residue definition corresponding to the MRLs residue definition in force (Reg. (EU) 2018/78).

According to the available toxicological information, a final conclusion about the toxicological relevance of the metabolite PHQ could not be reached. However, existing evidences clearly indicate that PHQ is more toxic than the parent OPP. In order to fulfil all the posibilities, two possible scenarios have provisionally been assessed:

Scenario 1:

Plant residue definition for monitoring (for fruit crops): 2-phenylphenol (sum of 2-phenylphenol and its conjugates, expressed as 2-phenylphenol).

Plant residue definition for risk assessment (for fruit crops): Sum of 2-phenylphenol and phenylhydroquinone and their salts and conjugates, expressed as 2-phenylphenol.

This residue definition for risk assessment fit with those risk assessment residues definitions which were proposed in the first active substance inclusion (EFSA Scientific Report (2008) 217, 1-67) and in the Review of the existing maximum residue levels for 2-phenylphenol according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal 2017; 15(1):4696). However since existing evidences clearly indicate that PHQ is more toxic than the parent OPP, alternative residue definition for risk assessment are proposed separately for OPP and PHQ (Scenario 2). In any case, it should be emphasized that the assessment about the toxicological relevance of the metabolite PHQ could not reach a final conclusion.

It must be recognized that PHQ has been found at very low levels (<0.2 mg/kg) in comparison with OPP for all the available residue trials in citrus fruits. Regarding definition for monitoring, we think that using the parent OPP could be sufficient since level of PHQ does not exceeed 10% of TRR in the metabolism studies and 0.2 mg/kg in the residue trials. Moreover, robust conversion factors (CF) from monitoring to risk assessment in whole fruits could be calculated.

Scenario 2:

Since existing evidences indicate that PHQ is more toxic than the parent OPP, residue definition for risk assessment could be proposed separately for OPP and PHQ:

Plant residue definition for monitoring (for fruit crops): 2-phenylphenol (sum of 2-phenylphenol and its conjugates, expressed as 2-phenylphenol).

Plant residue definition for risk assessment (for fruit crops) (2 separate residue definitions):
- Sum of 2-phenylphenol and their salts and conjugates, expressed as 2-phenylphenol.
- Sum of phenylhydroquinone and their salts and conjugates, expressed as phenylhydroquinone.

Animal residue definitions:

According to the results of the metabolism study, no residues are expected in animal commodities at the calculated dietary burden.

Since radioactive compound could not be identified form the available metabolisms study in goats (Thalacker, 1997) the parent compound is proposed by default and is applicable to ruminants based on the available data. During the art. 12 review an extrapolation to pigs was proposed on a tentative bases (EFSA Journal 2017;15(2):4696): "Since no metabolites could be identified in the metabolism study on ruminants due to the low residue levels found in milk and tissues, it was not possible to conclude whether the metabolism in rats and ruminants is similar. Consequently, the proposed residue definition for ruminants was extrapolated to pigs on a tentative basis only".

No residue definition for animal matrices was proposed in EFSA Scientific Report (2008) 217, 43-67.The residue definition for animal matrices is proposed to be parent compound by default (the same as in EFSA Journal 2017;15(2):4696):

Animal residue definition for monitoring: 2-phenylphenol.

Animal residue definition for risk assessment: 2-phenylphenol.

2.7.4 Summary of residue trials in plants and identification of critical GAP

OPP is proposed for use on citrus fruit according to the GAPs detailed in Table 2.7.4-1 to Table 2.7.4-3.

In addition the representative GAP (Table 2.7.4-1), which involves drenching application with AGF/1-04, GAPs are also shown for foam curtain application with AGF/1-03 (Table 2.7.4-2) and for wax application with AGC/1-10 (Table 2.7.4-3).

Table 2.7.4-1: Representative GAP for the Use of OPP on Citrus (Drenching Application)

EU country	Outdoor/ Indoor	Product name	Formulation concentration of a.s. (g/L)	Method kind	Growth stage (BBCH)	Max no. of apps.	Application rate per treatment (g a.s./hL)	PHI
Spain	Indoor	AGF/1-04 (EC)	100	Drencher	85-99	1	50 – 60	1

Table 2.7.4-2: Representative GAP for the Use of OPP on Citrus (Foam Curtain Application)

EU country	Outdoor/ Indoor	Product name	Formulation concentration of a.s. (g/L)	Method kind	Growth stage (BBCH)	Max no. of apps.	Application rate per treatment (g a.s./1000 kg fruit)	PHI
Spain	Indoor	AGF/1-03 (SL)	130 (13% w/v)	Foam curtain	85-99	1	26	1

Table 2.7.4-3: Representative GAP for the Use of OPP on Citrus (Wax Application)

EU country	Outdoor/ Indoor	Product name	Formulation concentration of a.s. (g/L)	Method kind	Growth stage (BBCH)	Max no. of apps.	Application rate per treatment (g a.s./1000 kg fruit)	PHI
Spain	Indoor	AGC/1-10 (EW)	2.5	Wax application	85-99	1	2.5	1

Residue data are presented on all three GAPs and products :
A total of 8 post-harvest residue trials on orange and 4 on mandarin are available for drenching application of AGF/1-04.
A total of 4 post-harvest residue trials on orange and 4 on mandarin are available for foam curtain application of AGF/1-03.
A total of 4 post-harvest residue trials on orange and 4 on mandarin are available for wax application of AGC/1-10.
All trials have been previously evaluated in the EU.
The results of the residue field trials are presented according to the Notifier´s proposed residue definitions for risk assessment and monitoring.

For an overview of available residue data please see table 2.7.4 7 below. **However, the Applicant is requested to provide justification on the independency of some trials, conducted in the same date, same location and, in some cases, same varieties, in order to consider them as independent (see vol. 3, point B.7.3.).**

Drench application:

Twelve post-harvest trials were conducted in Spain in between 2004 and 2010, 4 on mandarins and eight on oranges. Either AGF/1-04 (EC formulation containing 10% OPP) or CITROCIL (EC formulation containing 10% OPP and 7.5% imazalil) was applied to oranges or mandarins as a drench application at a rate of 60 g a.s./hL for 30 seconds in a closed drenching chamber.

Untreated orange and mandarin specimens were sampled directly before application while treated orange specimens were sampled 0 days, 7, 14 and 27-28 days after application (DAA) (except study 20044058/S1-FPOR: sampling only 0 DAA).

Most of the residue trials can be considered as valid and relevant. Residue levels of OPP and PHQ in whole fruits were analysed. However in study 20067012/S1-FPH (two trials), only residues in peel and pulp were analysed, and residues in whole fruit were calculated from weight ratios of peel and pulp to whole fruit. Storage stabilty was not validated for PHQ in fruit peel, and peel samples were frozen storaged 184 days before analysis for these two trials in study 20067012/S1-FPH.

Since the level of residues for PHQ in whole fruits is not detecteble for all the residue trials where it was directly analysed (8 trials), the level of residues in peel for this two residue trials (study 20067012/S1-FPH) is not foreseen to cause a higher level of PHQ residues in fruits. However, it must be recognized that most of the residue levels are in the peel of citrus fruits, and a reliable level of residues in whole fruits is therefore not calculated. These two residue trials should not be taken into account for PHQ.

Foam curtain application:

Eight post-harvest trials were conducted on mandarins (4 trials) and oranges (4 trials) in Spain in 2012 and 2013, respectively. In all trials AGF/1-03 (SL formulation containing 130 g OPP/L) was applied to citrus fruit as a foam curtain application at a rate of 26 g a.s./1000 kg fruit (±25%), as specified in the proposed GAP. The product was applied as a 10% product/water solution (100 mL of AGF/1-03 + 900 mL water).

Untreated mandarin and orange specimens were sampled directly before application while treated mandarin and orange specimens were sampled 0, 7, 13-14 and 27-28 days after application (DAA). Between application and sampling, the fruits were stored at a commercial storage house (in separate storage chambers) under chilled conditions. At sampling, specimens of whole orange fruits, orange pulp and orange peel were separated and deep-frozen (≤-18 °C) for a maximum of 43 (OPP) or 36 days (PHQ) before analysis.

All the residue trials can be considered as valid and relevant, residue levels of OPP and PHQ in whole fruits were analysed.

Waxing application:

Eight post-harvest trials were conducted on mandarins (4 trials) and oranges (4 trials) in Spain in 2012. In all trials AGC/1-10 (2.5 g OPP/L) was applied to citrus fruits as a wax application at a rate of 2.5 g a.s./1000 kg fruit, according to the proposed GAP. The product was applied directly without any dilution in a small scale pilot waxing plant.

Untreated mandarin and orange specimens were sampled directly before application while treated mandarin and orange specimens were sampled 0, 7, 14 ± 1 and 28 ± 1 days after application (DAA). Between application and sampling, the fruits were stored at a commercial storage house (separate storage chambers) under chilled conditions. After sampling, specimens of whole fruit, pulp and peel of both mandarins and oranges were deep-frozen (- 18°C) until analysis for a maximum of 77 (OPP) or 47 days (PHQ).

All the residue trials can be considered as valid and relevant, ~~r~~esidue levels of OPP and PHQ in whole fruits were analysed.

Overall summary (MRL calculation and Conversion factors for OPP residues after post-harvest application to citrus fruit):

An overview of the residue data and outputs from the OECD MRL calculator are shown in Table 2.7.4-7:

Residue data according to the residue definition for risk assessment were calculated from the sum of OPP plus PHQ, and using a conversion factor (CF) of 0.914 from OPP to PHQ (OPP 170.21 g/mol / PHQ 186.21 g/mol). In addition, all residue values that were below LOQ were assumed to be at the LOQ.

MRL values were calculated separately for drenching, foam curtain and wax application, but using the pooled data obtained for mandarin and orange to obtain sufficient data for MRL calculation.

The use of OPP at the trial GAPs leads to a calculated MRL of 4.0 mg/kg or less and is less than the existing EU MRL of 10.0 mg/kg (Regulation (EC) 2018/78). The existing EU MRL does not need to be amended.

A median Conversion factor (CF) for residue of OPP in citrus whole fruit from residue definition for enforcement to residue definition for risk assessment was calculated. The calculation was based on all available residue trials. The median CF is 1.30 (mean CF 1.35) (Table 2.7.4-8).

Table 2.7.4-7: Overview of All Available Residue Data after Post-Harvest Application of OPP to Citrus and Calculation of STMR, HR and MRL

Commodity	**Residue region, Outdoor/ Indoor**	**Product (type of application)**	**Individual trial results (mg/kg)** **E: Enforcement[a] & RA: Risk assessment[b]**	**STMR (mg/kg)**	**HR (mg/kg)**	**MRL[c] (mg/kg)**
Citrus	SEU, indoor (post-harvest)	AGF/1-04 (drencher)	Mandarin: E: 1.5, 2 x 1.9, 2.0 RA: 1.68, 2 x 2.08, 2.18	E. 1.90 RA: 2.08	E. 2.0 RA: 2.18	

Commodity	Residue region, Outdoor/ Indoor	Product (type of application)	Individual trial results (mg/kg)	STMR (mg/kg)	HR (mg/kg)	MRL[(c)] (mg/kg)
			E: Enforcement[(a)] & RA: Risk assessment[(b)]			
			Orange: E: 0.64, 0.67, 0.83, 1.10, 1.18, 1.30, 1.40, 1.60 RA: 0.82, 1.28, 1.36, 1.48, 1.58, 1.78	E. 1.14 RA: 1.42	E. 1.6 RA: 1.78	
			All data: E: 0.64, 0.67, 0.83, 1.10, 1.18, 1.30, 1.40, 1.5, 1.60, 2 x 1.9, 2.0 RA: 0.82, 1.28, 1.36, 1.48, 1.58, 1.68, 1.78, 2.08, 2.08, 2.18	E. 1.35 RA: 1.63	E. 2.0 RA: 2.18	4.0
		AGF/1-03 (foam curtain)	Mandarin: E. 0.40, 0.70, 2.10, 2.20 RA: 0.58, 0.88, 2.28, 2.38	E. 1.40 RA: 1.58	E. 2.20 RA: 2.38	
			Orange: E: 3 x 0.30, 0.60 RA: 3 x 0.48, 0.78	E. 0.30 RA: 0.48	E 0.60 RA: 0.78	
			All data: E: 3 x 0.30, 0.40, 0.60, 0.70, 2.10, 2.20 RA: 3 x 0.48, 0.58, 0.78, 0.88, 2.28, 2.38	E. 0.50 RA: 0.68	E. 2.20 RA: 2.38	4.0
		AGC/1-10 (waxing)	Mandarin: E: 0.48, 0.60, 0.64, 0.92 RA: 0.66, 0.78, 0.82, 1.10	E. 0.62 RA: 0.80	E. 0.92 RA: 1.10	
			Orange: E: 0.48, 0.69, 0.72, 1.08 RA: 0.66, 0.87, 0.90, 1.26	E. 0.71 RA: 0.89	E. 1.08 RA: 1.26	
			All data: E: 2 x 0.48, 0.60, 0.64, 0.69, 0.72, 0.92, 1.08 RA: 2 x 0.66, 0.78, 0.82, 0.87, 0.90, 1.10, 1.26	E. 0.67 RA: 0.85	E. 1.08 RA: 1.26	3.0

(a) Enforcement residue definition: 2-phenylphenol (sum of 2-phenylphenol and its conjugates, expressed as 2-phenylphenol) (Regulation (EU) 2018/78)

(b) Risk assessment residue definition: Sum of 2-phenylphenol and phenylhydroquinone and their salts and conjugates (EFSA, 2008). Calculated from OPP (mg/kg) + PHQ (mg/kg)*CF. CF calculated from MW OPP/MW PHQ = 170.21 g/mol / 186.21 g/mol = 0.914. Where PHQ was at n.d. or <LOQ a residue of 0.20 mg/kg was used for calculation.

(c) Calculated using the OECD method (ENV/JM/MONO(2011)3); rounded value.

Table 2.7.4-8: Conversion factors (CF) of Residues of OPP in Citrus Whole Fruit According to Residue Definition for Enforcement to Residue Definition for Risk Assessment

		Whole fruit			Conversion factor Residue definition for enforcement to residue definition for risk assessment [(c)]
Report No.; study No.	**DAA (days)**	**OPP[(a)] (mg/kg)**	**PHQ (mg/kg)**	**Sum of OPP+ PHQ[(b)] (mg/kg)**	
20044058/S1-FPMD S04W072R	0	1.2	<0.20	**1.38**	1.15
	7	1.8	<0.20	**1.98**	1.10

		Whole fruit			Conversion factor Residue definition for enforcement to residue definition for risk assessment [c]
Report No.; study No.	**DAA (days)**	**OPP[a] (mg/kg)**	**PHQ (mg/kg)**	**Sum of OPP+ PHQ[b] (mg/kg)**	
	14	1.9	<0.20	**2.08**	1.09
	28	1.5	<0.20	**1.68**	1.12
20044058/S1-FPMD S04W073R	0	1.2	<0.20	**1.38**	1.15
	7	1.6	<0.20	**1.78**	1.11
	14	2	<0.20	**2.18**	1.09
	28	1.6	<0.20	**1.78**	1.11
20044058/S1-FPMD S04W074R	0	1.4	<0.20	**1.58**	1.13
	7	1.2	<0.20	**1.38**	1.15
	14	1.5	<0.20	**1.68**	1.12
	28	1.4	<0.20	**1.58**	1.13
20044058/S1-FPMD S04W075R	0	1.3	<0.20	**1.48**	1.14
	7	1	<0.20	**1.18**	1.18
	14	1.4	<0.20	**1.58**	1.13
	28	1.9	<0.20	**2.08**	1.09
20044058/S1-FPOR S04W076R	0	1.4	<0.20	**1.58**	1.13
S11-01940-01	0	1.18	<0.20	**1.36**	1.15
	7	0.65	<0.20	**0.83**	1.28
	14	0.67	<0.20	**0.85**	1.27
	27	0.94; 0.82 = Mean 0.88	<0.20	**1.06**	1.20
S11-01940-02	0	0.47	<0.20	**0.65**	1.38
	7	0.46	<0.20	**0.64**	1.39
	14	0.41	<0.20	**0.59**	1.44
	27	0.67;0.60 = Mean 0.64	<0.20	**0.82**	1.28
S12-03980 S12-03980-01	0	2.2	<0.20	**2.38**	1.08
	7	0.4	<0.20	**0.58**	1.45
	14	0.7	<0.20	**0.88**	1.26
	28	0.4	<0.20	**0.58**	1.45
S12-03980 S12-03980-02	0	1.6	<0.20	**1.78**	1.11
	7	2.1	<0.20	**2.28**	1.09
	14	1	<0.20	**1.18**	1.18
	28	1.1	<0.20	**1.28**	1.16
S12-03980 S12-03980-03	0	0.5	<0.20	**0.68**	1.36
	7	0.6	<0.20	**0.78**	1.30
	14	0.7	<0.20	**0.88**	1.26
	28	0.3	<0.20	**0.48**	1.60
S12-03980 S12-03980-04	0	0.4	<0.20	**0.58**	1.45
	7	0.4	<0.20	**0.58**	1.45
	14	0.3	<0.20	**0.48**	1.60

		Whole fruit			Conversion factor Residue definition for enforcement to residue definition for risk assessment [c]
Report No.; study No.	**DAA (days)**	**OPP[a] (mg/kg)**	**PHQ (mg/kg)**	**Sum of OPP+ PHQ[b] (mg/kg)**	
	28	0.3	<0.20	**0.48**	1.60
S12-03980 S12-03980-05	0	0.6	<0.20	**0.78**	1.30
	7	0.5	<0.20	**0.68**	1.36
	13	0.5	<0.20	**0.68**	1.36
	27	0.4	<0.20	**0.58**	1.45
S12-03980 S12-03980-06	0	0.3	<0.20	**0.48**	1.60
	7	0.2	<0.20	**0.38**	1.90
	13	0.2	<0.20	**0.38**	1.90
	27	0.2	<0.20	**0.38**	1.90
S12-03980 S12-03980-07	0	0.3	<0.20	**0.48**	1.60
	7	0.3	<0.20	**0.48**	1.60
	13	0.2	<0.20	**0.38**	1.90
	27	0.2	<0.20	**0.38**	1.90
S12-03980 S12-03980-08	0	0.3	<0.20	**0.48**	1.60
	7	0.3	<0.20	**0.48**	1.60
	13	0.23	<0.20	**0.41**	1.78
	27	0.2	<0.20	**0.38**	1.90
S11-03862 S12-03862-01	0	0.6	<0.20	**0.78**	1.30
	7	0.48	<0.20	**0.66**	1.38
	13	0.57	<0.20	**0.75**	1.32
	28	0.46	<0.20	**0.64**	1.39
S11-03862 S12-03862-02	0	0.53	<0.20	**0.71**	1.34
	7	0.54	<0.20	**0.72**	1.33
	14	0.92	<0.20	**1.1**	1.20
	28	0.85	<0.20	**1.03**	1.21
S11-03862 S12-03862-03	0	0.36	<0.20	**0.54**	1.50
	7	0.48	<0.20	**0.66**	1.38
	14	0.4	<0.20	**0.58**	1.45
	27	0.35	<0.20	**0.53**	1.51
S11-03862 S12-03862-04	0	0.64	<0.20	**0.82**	1.28
	7	0.49	<0.20	**0.67**	1.37
	14	0.51	<0.20	**0.69**	1.35
	28	0.58	<0.20	**0.76**	1.31
S11-03862 S12-03862-05	0	0.41	<0.20	**0.59**	1.44
	7	0.68	<0.20	**0.86**	1.26
	14	0.72	<0.20	**0.9**	1.25
	28	0.71	<0.20	**0.89**	1.25
S11-03862 S12-03862-06	0	1.08	<0.20	**1.26**	1.17
	7	0.85	<0.20	**1.03**	1.21
	14	1.02	<0.20	**1.2**	1.18
	28	1.01	<0.20	**1.19**	1.18
S11-03862	0	0.63	<0.20	**0.81**	1.29

		Whole fruit			Conversion factor Residue definition for enforcement to residue definition for risk assessment [c]
Report No.; study No.	**DAA (days)**	**OPP[a] (mg/kg)**	**PHQ (mg/kg)**	**Sum of OPP+ PHQ[b] (mg/kg)**	
S12-03862-08	7	0.69	<0.20	**0.87**	1.26
	13	0.57	<0.20	**0.75**	1.32
	29	0.63	<0.20	**0.81**	1.29
S11-03862	0	0.48	<0.20	**0.66**	1.38
S12-03862-09	7	0.38	<0.20	**0.56**	1.47
	13	0.45	<0.20	**0.63**	1.40
	29	0.36	<0.20	**0.54**	1.50
				Median	**1.30**
				Mean	**1.35**
				Min	**1.08**
				Max	**1.90**

(a) Residue according to residue definition for enforcement (sum of OPP and its conjugates, expressed as OPP)
(b) Sum of OPP and PHQ = OPP (mg/kg) + PHQ (mg/kg) * 0.914. MW adjustment 0.914 calculated from MW OPP/MW PHQ = 170.21 g/mol / 186.21 g/mol;
If the residue of PHQ was <LOQ, this value was treated as "at LOQ"
(c) Conversion factor for the sum of OPP and PHQ (and their conjugates) (as OPP) in whole fruit according to residue definition for risk assessment / OPP (and its conjugates) in whole fruit according to the residue definition for enforcement

2.7.5 Summary of feeding studies in poultry, ruminants, pigs and fish

According to the dietary burden calculation (Animal model, 2017) the trigger level of 0.004 mg/kg bw/day is exceeded for cattle (dairy and meat) and breeding swine.

However it should be emphasized that the intended uses are post-harvest uses for citrus fruits. Citrus dried pulp constitutes of the combination of the remaining pulp and peel after drying of the by-product of the juicing process. In the common industrial practice, the citrus fruits used for processing into juice are not treated with OPP, and therefore OPP should not be present in citrus processing products intended for animal feed.

The only relevant feed commodity for the intended uses of OPP is the citrus dried pulp. Citrus dried pulp is a feed item only relevant to cattle and breeding swine. Therefore, feeding studies on poultry are not required.

According to the dietary burden calculation (Animal model, 2017) the trigger level of 0.004 mg/kg bw/day is exceeded for cattle (dairy and meat).

However it should be emphasized that the intended uses are post-harvest uses for citrus fruits. In the common industrial practice, the citrus fruits used for processing into juice are not treated with OPP, and therefore OPP should not be present in citrus processing products intended for animal feed. Motreover according to the results of the metabolism study, no residues are expected in animal commodities at the calculated dietary burden. Therefore, the runinants feeding study is not considered as essential bearind in mind the current post-harvest uses in citrus fruits.

Although metabolites were not identified in lactating goats, it does not become apparent that metabolic pathways differ significantly in the rat as compared to ruminants. Hence it is safely assumed that OPP metabolism for pigs follows a similar pattern as for ruminants, and most of the residues will be excreted via urine and faeces within 24 hours of dose administration. According to Commission Regulation (EU) No. 283/2013, metabolism studies on pigs are necessary where it becomes apparent that metabolic pathways differ significantly in the rat as compared to ruminants. Since it does not seem the case, feeding studies in pigs are not considered to be necessary according to the intended uses.

According to SANCO 11187/2013, citrus fruit and their processing products are not considered as commodities commonly used for the formulation of aquaculture diets (see Annex 2. Feedingstuffs table). Therefore, the use of

OPP according to the intended uses is not foreseen to affect fishes feeding, feeding studies for fishes are not necessary.

2.7.6 Summary of effects of processing

A study was performed to determine the effects of different heating conditions, to simulate different process, on OPP. The standard conditions were representative of pasteurisation (pH 4, 90°C, 20 minutes), baking/boiling/brewing (pH 5, 100°C, 60 minutes) and sterilisation (pH 6, 120°C, 20 minutes). The results indicate that OPP is stable under the three standard processing conditions.

It should be reminded that, a loss of approximately 15% was found in the experiment simulating sterilisation; however no metabolites were detected. Nevertheless, the PRAPeR 60 meeting concluded that no breakdown of 2-phenylphenol was observed and that the compound could be regarded as stable under the conditions studied. Since OPP showed to be stable following standard processing conditions, the same residue definition for raw citrsu fruits applies also to processed commodities.
A summary of the findings is given below.

Table 2.7.6-1: Summary of Nature of the Residues in Processed Commodities

Conditions (Duration, Temperature, pH)	Identified compound(s) (%)	Reference
Pasteurisation (20 minutes, 90°C, pH 4)	Parent (100%)	Morlock, G. (2005) EU agreed (Spain, 2008)
Baking, boiling, brewing (60 minutes, 100°C, pH 5)	Parent (100%)	
Sterilisation (20 minutes, 120°C, pH 6)	Parent (100%)	

The distribution of residues of OPP in inedible peel and pulp is relevant to post-harvest applications to citrus fruits. In 23 of the residue trials presented, samples of whole fruit were separated into peel and pulp and analysed for residues of OPP and PHQ and their conjugates. No residues were detected in pulp above the relevant LOQ values for OPP and PHQ, 0.10 mg/kg and 0.20 mg/kg respectively.
Transfers factors were calculated according to the residue definition for enforcement and according to the residue definition for risk assessment separately, for both, the ratio of peel to whole fruit and for the ratio of pulp to whole fruit.

Since no residues of OPP or PHQ were detected in citrus pulp, the mean transfer factor of residues in pulp to residues in whole fruit is <0.38 (median <0.36) according to the residue definition for risk assessment, and <0.19 (median <0.17) according to the residue definition for enforcement.
Most of the residue of OPP and PHQ was concentrated in the peel. For enforcement purposes, the mean transfer factor of residues in peel to residues in whole fruit was calculated to be 3.79 (median 3.36).
For risk assessment purposes, a mean transfer factor of residues in peel to residues in whole fruit of 3.04 (median 2.88) was calculated. However, since storage stability for PHQ in peel is not validated, the figures of PHQ are not reliable, and this transfer factor of residues in whole fruit to residues in peel is not robust for risk assessment.

Three processing studies have been conducted in oranges. An overview of all available studies is given in the table below.

Table 2.7.6 -2: Summary of the available processing studies

Processed commodity	Number of studies	Median PF *	Median CF **	Comments	Reference
Enforcement residue definition: 2-phenylphenol (sum of 2-phenylphenol and its conjugates, expressed as 2-phenylphenol)					
Orange, marmalade	4	0.36	1.20		Pollmann, B. (2005b) EU agreed (Spain, 2008)
Orange, dry pomace	1[(a)]	3.8	0.98	-	Johnson, G.D., Strickland, M.D.,
Orange, juice	1[(a)]	0.04	1.50	-	

Processed commodity	Number of studies	Median PF *	Median CF **	Comments	Reference
Orange, oil	1[(a)]	84	0.98	-	1996 EU agreed (Spain, 2008)
Orange, dry pomace	2	0.11	1.24	-	Gonzalez, J.B. (2019) New data
Orange, juice	2	0.04	1.57	-	
Orange, oil	2	37	0.98	-	

* The median processing factor is obtained by calculating the median of the individual processing factors of each processing study.

** The median conversion factor for enforcement to risk assessment is obtained by calculating the median of the individual conversion factors of each processing study.

(a) One study in which two replicate samplings were taken at DAA 0, 28 and 56, each.

Study 20044058/S1-FPOR was well performed and reported. Four processing studies with oranges treated post-harvest with AGF/1-04 as a drencher application of 60 g a.s./hL was conducted in 2004 in Spain. The mean transfer factor calculated for orange marmalade was 0.43 according to the residue definition for risk assessment, and 0.36 according to the residue definition for monitoring, indicating that the residue is not concentrated with respect to the RAC whole fruit. Conversion factors (CF) for marmalade for the TF monitoring to TF risk assessment ranged from 1.16 to 1.38, with a mean CF of 1.23 (median CF 1.20). Transfer factors for OPP in washed fruit, washing water and pulp were also below 1, indicating the residue is not concentrated in theses matrices. OPP was concentrated in peel, with a transfer factor of 3.22 (risk assessment residue definition) and 3.5 (monitoring residue definition).
Pollmann, B. (2005) validation of Harsy, S. G. GC-MS method is acceptable and shall support the residue study 20044058/S1-FPMD in orange processed fractions.

A processing study (CCQC 94-05) with oranges treated post-harvest with sodium orthophenylphenate (SOPP) was conducted in 1995 in USA. SOPP was applied at an exaggerated rate. Residues of OPP were determined in the raw agricultural commodity (RAC), and in juice, dry pomace and oil. Determinations of the metabolite PHQ were performed in the same matrices except in dry pomace because of degradation during the processing procedure. Results for PHQ in orange oil were analised but not reported because the storage stability of PHQ in oil samples is not demonstrated. Results of OPP in orange oil are not reliable too since frozen storage period from sampling to extraction (235 days) is clearly higher than the tested period (100 days)

The mean transfer factors calculated according to the residue definition for monitoring were 3.6 for dry pomace, 0.03 for orange juice and 84 for orange oil.
The mean conversion factors (CF) for TF monitoring to TF risk assessment were 0.98, 1.51 and 0.98 for dry pomace, juice and oil, respectively. However, since values for OPP and PHQ in oil are not reliable, the calculated mean conversion factors (CF) for TF monitoring to TF risk assessment for oil are not reliable too.
Harsy, S. G. (1996) GC-MS method used in the validated analytical study proves to measure OPP, PHQ and their conjugates with reasonable accuracy. It is acceptable.

Two processing trials (S18-02441) with oranges treated post-harvest with AGF/1-04 as a drencher application at the exaggerated rate of 300 g a.s./hL was conducted in 2018 in Spain.
Oranges were stored in commercial storage for 7 days. They were then processed into juice, dry pomace and oil according to processes used for commercial purposes, and analysed for OPP and PHQ according to a method validated within the report. Results for PHQ in orange oil are not reported because PHQ is not stable in oil.
The mean transfer factors calculated according to the residue definition for monitoring were 0.11 for dry pomace, 0.04 for pasteurized orange juice and 37 for orange oil.
Mean conversion factors (CF) for monitoring to risk assessment 1.24, 1.57 and 0.98 for dry pomace, juice and oil, respectively. However, since PHQ in oil is not stable during frozen storage, mean conversion factors (CF) for monitoring to risk assessment for oil is not reliable.
Driss, F. (2019) validated LC-MS/MS method is considered acceptable and supportive for the residue study of OPP and PHQ in orange processed fractions.

2.7.7 Summary of residues in rotational crops

Residues in rotational crops are not relevant for post-harvest applications. RMS agrees with Notifier´s rationale.

2.7.8 Summary of other studies

Effects on the residue level in pollen and bee products are not relevant since product is used post-harvest.

In accordance with Regulation (EU) 283/2013, a summary of all relevant data from the scientific peer reviewed open literature on the active substance, metabolites and breakdown or reaction products and plant protection product containing the active substance has been conducted. A total of 11 references have been identified as relevant to the risk areas of toxicology, environmental fate and ecotoxicology and have been reviewed in detail for potential relevance to the risk assessments of OPP and its metabolite. However, not any relevant reference was found for Residues Section.

2.7.9 Estimation of the potential and actual exposure through diet and other sources

According to the available toxicological information, an assessment about the toxicological relevance of the metabolite PHQ could not be finished. However, existing evidences clearly indicate that PHQ is more toxic than the parent OPP. In order to fulfil all the posibilties, two possible scenarios have been assessed:

Exposure calculations for OPP were done using the EFSA PRIMo 3.1[8] version of the model.

Scenario 1:
For this scenario, the risk assessment residue definition was considered as sum of 2-phenylphenol and phenylhydroquinone and their salts and conjugates, expressed as 2-phenylphenol:

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation:

The ADI for OPP has been set at 0.4 mg/kg (EFSA, 2008) and same value is proposed for the assessment of the renewal of the approval

The TMDI was calculated according to the refined calculation mode, using current EU MRLs for the uses proposed in this document and in commodities of animal origin. Input values are shown in Table 2.7.9-1.

Details of the TMDI calculation are shown in Table 2.7.9-2. The highest exposure is for the DE child diet at 16% of the ADI, with oranges contributing 13%. The long-term estimated dietary intake is therefore below the ADI and a risk to the consumer is unlikely.

Table 2.7.9-1: Input values for OPP assessment in citrus fruits

Commodity	Chronic risk assessment	
	Input value (mg/kg)	Comment
Risk assessment residue definition : Sum of 2-phenylphenol and phenylhydroquinone and their salts and conjugates, expressed as 2-phenylphenol		
Grapefruits (110010)	13.0	EU MRL[(a)] x CF (10 mg/kg x 1.30; CF see Section CA 6.3.1)
Oranges (110020)	13.0	EU MRL[(a)] x CF (10 mg/kg x 1.30; CF see Section CA 6.3.1)

[8] EFSA (European Food Safety Authority), 2019. Pesticide Residue Intake Model - EFSA PRIMo revision 3.1 (update of EFSA PRIMo revision 3). EFSA supporting publication 2019:EN-1605. 15 pp. doi:10.2903/sp.efsa.2019.EN-160
Excel spreadsheet at https://www.efsa.europa.eu/de/applications/pesticides/tools

Commodity	Chronic risk assessment	
	Input value (mg/kg)	Comment
Lemons (110030)	13.0	EU MRL[(a)] x CF (10 mg/kg x 1.30; CF see Section CA 6.3.1)
Limes (110040)	13.0	EU MRL[(a)] x CF (10 mg/kg x 1.30; CF see Section CA 6.3.1)
Mandarins (110050)	13.0	EU MRL[(a)] x CF (10 mg/kg x 1.30; CF see Section CA 6.3.1)
Other citrus fruit (110990)	13.0	EU MRL[(a)] x CF (10 mg/kg x 1.30; CF see Section CA 6.3.1)
Products of animal origin – tissues (1010000)	0.01	EU MRL at LOQ[(a)]
Products of animal origin – milk (1020000)	0.01	EU MRL at LOQ[(a)]
Products of animal origin – birds eggs (1030000)	0.01	EU MRL at LOQ[(a)]

(a) EU MRL Regulation (EC) 2018/78

Scenario 2:

For this scenario, different residue definition for OPP and PHQ has been considered since existing evidences indicate that PHQ is more toxic than the parent OPP:

- Sum of 2-phenylphenol and their salts and conjugates expressed as 2-phenylphenol.
- Sum of phenylhydroquinone and their salts and conjugates expressed as phenylhydroquinone.

The ADI for OPP has been set at 0.4 mg/kg, whilst for PHQ the value proposed by the Notifier (0.045 mg/kg bw/day has been used tentatively for the calculation (awaiting more conclusive data).

Chronic risk assessment for OPP:

The ADI for OPP has been used in the calculation (EFSA PRIMo 3.1)

The TMDI was calculated according to the refined calculation mode, using current EU MRLs for the uses proposed in this document and in commodities of animal origin. Input values are shown in Table 2.7.9-3.

Details of the TMDI calculation are shown in Table 2.7.9-4. The highest exposure is for the DE child diet at 12% of the ADI, with oranges contributing 10%. The long-term estimated dietary intake is therefore below the ADI and a risk to the consumer is unlikely.

Table 2.7.9-3: Input values for OPP assessment in citrus fruits

Commodity	Chronic risk assessment	
	Input value (mg/kg)	Comment
Risk assessment residue definition : Sum of 2-phenylphenol and their salts and conjugates, expressed as 2-phenylphenol		
Grapefruits (110010)	10.0	EU MRL[(a)]
Oranges (110020)	10.0	EU MRL[(a)]
Lemons (110030)	10.0	EU MRL[(a)]
Limes (110040)	10.0	EU MRL[(a)]
Mandarins (110050)	10.0	EU MRL[(a)]

Commodity	Chronic risk assessment	
	Input value (mg/kg)	**Comment**
Other citrus fruit (110990)	10.0	EU MRL[(a)]
Products of animal origin – tissues (1010000)	0.01	EU MRL at LOQ[(a)]
Products of animal origin – milk (1020000)	0.01	EU MRL at LOQ[(a)]
Products of animal origin – birds eggs (1030000)	0.01	EU MRL at LOQ[(a)]

(a) EU MRL Regulation (EC) 2018/78

Chronic risk assessment for PHQ:

The ADI for PHQ could not be concluded; however, it seem to be clear that PHQ is more toxic than OPP. Tentatively, the Notifier´s proposal for an ADI of 0.045 mg/kg bw/day has been used in the calculation (EFSA PRIMo 3.1)

The TMDI was calculated according to the refined calculation mode, using the highest value of PHQ from the available residue trials (0.2 mg/kg). Regarding commodities of animal origin, according to the livestock metabolism assessment, significant level of residues is not foreseen for animal origin commodities. Since not analytical method is available for PHQ in livestock origin commodities, LOQ can not be incorporated to the calculation. Input values are shown in Table 2.7.9-5.

Details of the TMDI calculation are shown in Table 2.7.9-6. The highest exposure is for the DE child and FR child (3-15 years) diets at 2% of the provisional ADI. The long-term estimated dietary intake is therefore by far below the tentative ADI and a risk to the consumer is unlikely for PHQ.

Table 2.7.9-5: Input values used for PHQ assessment in citrus fruits

Commodity	Chronic risk assessment	
	Input value (mg/kg)	**Comment**
Risk assessment residue definition : Sum of phenylhydroquinone and their salts and conjugates, expressed as phenylhydroquinone		
Grapefruits (110010)	0.2	Highest value from the available residue trials
Oranges (110020)	0.2	Highest value from the available residue trials
Lemons (110030)	0.2	Highest value from the available residue trials
Limes (110040)	0.2	Highest value from the available residue trials
Mandarins (110050)	0.2	Highest value from the available residue trials
Other citrus fruit (110990)	0.2	Highest value from the available residue trials
Products of animal origin – tissues (1010000)	-	Not analytical method available
Products of animal origin – milk (1020000)	-	Not analytical method available
Products of animal origin – birds eggs (1030000)	-	Not analytical method available (not LOQ available)

Acute Reference Dose (ARfD) and Dietary Exposure Calculation:

According to EFSA (2008) an ARfD is not required, and has not been set by RMS in the assessment for the renewal of the approval. An acute risk assessment calculation has therefore not been performed.

Table 2.7.9-2: Scenario 1: TMDI calculations (EFSA PRIMo 3.1): sum of 2-phenylphenol and phenylhydroquinone and their salts and conjugates, expressed as 2-phenylphenol

European Food Safety Authority

EFSA PRIMo revision 3.1; 2019/03/19

2-phenylphenol

LOQs (mg/kg) range from: to:

Toxicological reference values

ADI (mg/kg bw/day):	**0.4**	ARfD (mg/kg bw):	**not necessary**
Source of ADI:	**EFSA**	Source of ARfD:	**EFSA**
Year of evaluation:	**2008**	Year of evaluation:	**2008**

Input values

Details - chronic risk assessment | Supplementary results - chronic risk assessment

Details - acute risk assessment/children | Details - acute risk assessment/adults

Comments:

Refined calculation mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI : ---

	Calculated exposure (% of ADI)	MS Diet	Expsoure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from: MRLs set at the LOQ (in % of ADI)	Exposure resulting from: commodities not under assessment (in % of ADI)
TMDI/NEDI/IEDI calculation (based on average food consumption)	16%	DE child	62.89	13%	Oranges	1%	Mandarins	0.7%	Grapefruits		16%
	12%	FR child 3 15 yr	47.36	11%	Oranges	0.5%	Mandarins	0.1%	Grapefruits		12%
	9%	NL toddler	37.80	7%	Oranges	1%	Mandarins	0.4%	Lemons		9%
	8%	NL child	30.73	5%	Oranges	2%	Mandarins	0.6%	Lemons		8%
	8%	ES child	30.62	7%	Oranges	0.5%	Mandarins	0.0%	Milk: Cattle		8%
	8%	IE adult	30.46	3%	Oranges	2%	Grapefruits	2%	Mandarins		8%
	8%	FR toddler 2 3 yr	30.45	5%	Oranges	3%	Mandarins	0.3%	Grapefruits		8%
	8%	DE women 14-50 yr	30.43	6%	Oranges	0.7%	Lemons	0.3%	Mandarins		8%
	8%	UK toddler	30.24	6%	Oranges	0.9%	Mandarins	0.1%	Grapefruits		8%
	6%	DE general	25.40	5%	Oranges	0.7%	Lemons	0.3%	Grapefruits		6%
	6%	GEMS/Food G07	24.89	5%	Oranges	0.7%	Mandarins	0.5%	Lemons		6%
	5%	GEMS/Food G06	21.38	3%	Oranges	1%	Mandarins	0.9%	Lemons		5%
	5%	GEMS/Food G10	21.05	4%	Oranges	0.7%	Lemons	0.6%	Mandarins		5%
	5%	GEMS/Food G11	20.96	2%	Oranges	1%	Lemons	1%	Grapefruits		5%
	5%	ES adult	18.90	4%	Oranges	0.5%	Mandarins	0.0%	Lemons		5%
	5%	SE general	18.22	2%	Oranges	1%	Mandarins	0.3%	Grapefruits		5%
	4%	UK infant	17.90	4%	Oranges	0.1%	Milk: Cattle	0.1%	Grapefruits		4%
	4%	NL general	16.54	3%	Oranges	0.6%	Mandarins	0.2%	Grapefruits		4%
	3%	GEMS/Food G08	13.73	1%	Oranges	0.8%	Lemons	0.8%	Mandarins		3%
	3%	UK vegetarian	13.68	3%	Oranges	0.4%	Grapefruits	0.2%	Mandarins		3%
	3%	GEMS/Food G15	12.79	2%	Oranges	0.5%	Mandarins	0.3%	Lemons		3%
	2%	PT general	9.97	2%	Oranges	0.3%	Mandarins	0.2%	Lemons		2%
	2%	IT toddler	9.59	2%	Oranges	0.7%	Mandarins	0.1%	Lemons		2%
	2%	FR adult	9.43	2%	Oranges	0.2%	Mandarins	0.2%	Grapefruits		2%
	2%	UK adult	9.09	2%	Oranges	0.2%	Grapefruits	0.2%	Mandarins		2%
	2%	IT adult	7.53	1%	Oranges	0.5%	Mandarins	0.1%	Lemons		2%
	2%	FI adult	7.33	1%	Oranges	0.5%	Mandarins	0.0%	Grapefruits		2%
	2%	FI 3 yr	7.32	1%	Mandarins	0.5%	Oranges	0.1%	Grapefruits		2%
	2%	FI 6 yr	6.41	1%	Mandarins	0.5%	Oranges	0.0%	Grapefruits		2%
	1%	RO general	5.78	0.9%	Oranges	0.2%	Grapefruits	0.2%	Grapefruits		1%
	1%	FR infant	5.71	0.8%	Oranges	0.5%	Mandarins	0.1%	Grapefruits		1%
	1%	DK child	4.27	0.6%	Oranges	0.3%	Mandarins	0.1%	Grapefruits		1%
	0.9%	DK adult	3.69	0.5%	Oranges	0.4%	Mandarins	0.1%	Grapefruits		0.9%
	0.4%	PL general	1.66	0.2%	Lemons	0.1%	Mandarins	0.1%	Oranges		0.4%
	0.3%	LT adult	1.37	0.2%	Oranges	0.0%	Mandarins	0.0%	Lemons		0.3%
	0.3%	IE child	1.26	0.3%	Oranges	0.0%	Grapefruits	0.0%	Lemons		0.3%

Conclusion:

The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.

The long-term intake of residues of 2-phenylphenol is unlikely to present a public health concern.

Table 2.7.9-4: Scenario 2: TMDI calculations (EFSA PRIMo 3.1): sum of 2-phenylphenol and their salts and conjugates, expressed as 2-phenylphenol

European Food Safety Authority

EFSA PRIMo revision 3.0; 2017/12/11

OPP

LOQs (mg/kg) range from: to:

Toxicological reference values

ADI (mg/kg bw/day):	**0,4**	ARfD (mg/kg bw):	**not necessary**
Source of ADI:		Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Input values

Details - chronic risk assessment | Supplementary results - chronic risk assessment

Details - acute risk assessment/children | Details - acute risk assessment/adults

Comments:

Normal mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI : ---

TMDI/NEDI/IEDI calculation (based on average food consumption)	Calculated exposure (% of ADI)	MS Diet	Expsoure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from MRLs set at the LOQ (in % of ADI)	Exposure resulting from commodities not under assessment (in % of ADI)
	12%	DE child	48,43	10%	Oranges	1%	Mandarins	0,6%	Grapefruits		12%
	9%	FR child 3 15 yr	36,49	9%	Oranges	0,4%	Mandarins	0,1%	Grapefruits		9%
	7%	NL toddler	29,22	6%	Oranges	1%	Mandarins	0,3%	Lemons		7%
	6%	NL child	23,70	4%	Oranges	2%	Mandarins	0,4%	Lemons		6%
	6%	ES child	23,59	5%	Oranges	0,4%	Mandarins	0,0%	Milk: Cattle		6%
	6%	FR toddler 2 3 yr	23,50	4%	Oranges	2%	Mandarins	0,2%	Grapefruits		6%
	6%	IE adult	23,45	3%	Oranges	2%	Grapefruits	1%	Mandarins		6%
	6%	DE women 14-50 yr	23,44	5%	Oranges	0,6%	Lemons	0,3%	Mandarins		6%
	6%	UK toddler	23,32	5%	Oranges	0,7%	Mandarins	0,1%	Grapefruits		6%
	5%	DE general	19,57	4%	Oranges	0,5%	Lemons	0,2%	Grapefruits		5%
	5%	GEMS/Food G07	19,17	3%	Oranges	0,5%	Mandarins	0,4%	Lemons		5%
	4%	GEMS/Food G06	16,46	2%	Oranges	0,8%	Mandarins	0,7%	Lemons		4%
	4%	GEMS/Food G10	16,21	3%	Oranges	0,5%	Lemons	0,4%	Mandarins		4%
	4%	GEMS/Food G11	16,15	2%	Oranges	0,9%	Lemons	0,8%	Grapefruits		4%
	4%	ES adult	14,56	3%	Oranges	0,4%	Mandarins	0,0%	Lemons		4%
	4%	SE general	14,06	2%	Oranges	1%	Mandarins	0,3%	Grapefruits		4%
	3%	UK infant	13,87	3%	Oranges	0,1%	Milk: Cattle	0,1%	Grapefruits		3%
	3%	NL general	12,75	3%	Oranges	0,4%	Mandarins	0,2%	Grapefruits		3%
	3%	GEMS/Food G08	10,58	1%	Oranges	0,7%	Lemons	0,6%	Mandarins		3%
	3%	UK vegetarian	10,53	2%	Oranges	0,3%	Grapefruits	0,1%	Mandarins		3%
	2%	GEMS/Food G15	9,86	2%	Oranges	0,4%	Mandarins	0,3%	Lemons		2%
	2%	PT general	7,67	2%	Oranges	0,2%	Mandarins	0,1%	Lemons		2%
	2%	IT toddler	7,38	1%	Oranges	0,5%	Mandarins	0,1%	Lemons		2%
	2%	FR adult	7,27	1%	Oranges	0,2%	Mandarins	0,1%	Grapefruits		2%
	2%	UK adult	7,01	1%	Oranges	0,2%	Grapefruits	0,1%	Mandarins		2%
	1%	IT adult	5,79	0,9%	Oranges	0,4%	Mandarins	0,1%	Lemons		1%
	1%	FI adult	5,64	1%	Oranges	0,4%	Mandarins	0,0%	Grapefruits		1%
	1%	FI 3 yr	5,63	1,0%	Mandarins	0,4%	Oranges	0,1%	Grapefruits		1%
	1%	FI 6 yr	4,93	0,8%	Mandarins	0,4%	Oranges	0,0%	Grapefruits		1%
	1%	RO general	4,48	0,7%	Oranges	0,1%	Grapefruits	0,1%	Grapefruits		1%
	1%	FR infant	4,43	0,6%	Oranges	0,4%	Mandarins	0,1%	Grapefruits		1%
	0,8%	DK child	3,32	0,5%	Oranges	0,2%	Mandarins	0,1%	Grapefruits		0,8%
	0,7%	DK adult	2,86	0,4%	Oranges	0,3%	Mandarins	0,0%	Grapefruits		0,7%
	0,3%	PL general	1,27	0,2%	Lemons	0,1%	Mandarins	0,0%	Oranges		0,3%
	0,3%	LT adult	1,06	0,2%	Oranges	0,0%	Mandarins	0,0%	Lemons		0,3%
	0,2%	IE child	0,98	0,2%	Oranges	0,0%	Grapefruits	0,0%	Lemons		0,2%

Conclusion:
The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.
The long-term intake of residues of OPP is unlikely to present a public health concern.

Table 2.7.9-6: Scenario 2: Tentative TMDI calculations (EFSA PRIMo 3.1): sum of phenylhydroquinone and their salts and conjugates, expressed as phenylhydroquinone

European Food Safety Authority

EFSA PRIMo revision 3.0; 2017/12/11

PHQ

LOQs (mg/kg) range from: to:

Toxicological reference values

ADI (mg/kg bw/day):	**0,045**	ARfD (mg/kg bw):	**not necessary**
Source of ADI:		Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Input values

Details - chronic risk assessment | Supplementary results - chronic risk assessment

Details - acute risk assessment/children | Details - acute risk assessment/adults

Comments:

Normal mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI : ---

TMDI/NEDI/IEDI calculation (based on average food consumption)	Calculated exposure (% of ADI)	MS Diet	Expsoure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from MRLs set at the LOQ (in % of ADI)	Exposure resulting from commodities not under assessment (in % of ADI)
	2%	DE child	0,96	2%	Oranges	0,2%	Mandarins	0,1%	Grapefruits		2%
	2%	FR child 3 15 yr	0,72	2%	Oranges	0,1%	Mandarins	0,0%	Grapefruits		2%
	1%	NL toddler	0,57	1,0%	Oranges	0,2%	Mandarins	0,1%	Lemons		1%
	1%	NL child	0,47	0,6%	Oranges	0,3%	Mandarins	0,1%	Lemons		1%
	1%	ES child	0,47	1,0%	Oranges	0,1%	Mandarins	0,0%	Lemons		1%
	1%	IE adult	0,47	0,5%	Oranges	0,3%	Grapefruits	0,2%	Mandarins		1%
	1%	DE women 14-50 yr	0,47	0,8%	Oranges	0,1%	Lemons	0,0%	Mandarins		1%
	1%	FR toddler 2 3 yr	0,46	0,6%	Oranges	0,3%	Mandarins	0,0%	Grapefruits		1%
	1%	UK toddler	0,46	0,9%	Oranges	0,1%	Mandarins	0,0%	Grapefruits		1%
	0,9%	DE general	0,39	0,7%	Oranges	0,1%	Lemons	0,0%	Grapefruits		0,9%
	0,8%	GEMS/Food G07	0,38	0,6%	Oranges	0,1%	Mandarins	0,1%	Lemons		0,8%
	0,7%	GEMS/Food G06	0,33	0,4%	Oranges	0,1%	Mandarins	0,1%	Lemons		0,7%
	0,7%	GEMS/Food G10	0,32	0,5%	Oranges	0,1%	Lemons	0,1%	Mandarins		0,7%
	0,7%	GEMS/Food G11	0,32	0,3%	Oranges	0,2%	Lemons	0,1%	Grapefruits		0,7%
	0,6%	ES adult	0,29	0,6%	Oranges	0,1%	Mandarins	0,0%	Lemons		0,6%
	0,6%	SE general	0,28	0,3%	Oranges	0,2%	Mandarins	0,0%	Grapefruits		0,6%
	0,6%	UK infant	0,27	0,6%	Oranges	0,0%	Grapefruits	0,0%	Grapefruits		0,6%
	0,6%	NL general	0,25	0,4%	Oranges	0,1%	Mandarins	0,0%	Grapefruits		0,6%
	0,5%	UK vegetarian	0,21	0,4%	Oranges	0,1%	Grapefruits	0,0%	Mandarins		0,5%
	0,5%	GEMS/Food G08	0,21	0,2%	Oranges	0,1%	Lemons	0,1%	Mandarins		0,5%
	0,4%	GEMS/Food G15	0,20	0,3%	Oranges	0,1%	Mandarins	0,0%	Lemons		0,4%
	0,3%	PT general	0,15	0,3%	Oranges	0,0%	Mandarins	0,0%	Lemons		0,3%
	0,3%	IT toddler	0,15	0,2%	Oranges	0,1%	Mandarins	0,0%	Lemons		0,3%
	0,3%	FR adult	0,14	0,3%	Oranges	0,0%	Mandarins	0,0%	Grapefruits		0,3%
	0,3%	UK adult	0,14	0,2%	Oranges	0,0%	Grapefruits	0,0%	Mandarins		0,3%
	0,3%	IT adult	0,12	0,2%	Oranges	0,1%	Mandarins	0,0%	Lemons		0,3%
	0,3%	FI adult	0,11	0,2%	Oranges	0,1%	Mandarins	0,0%	Grapefruits		0,3%
	0,3%	FI 3 yr	0,11	0,2%	Mandarins	0,1%	Oranges	0,0%	Grapefruits		0,3%
	0,2%	FI 6 yr	0,10	0,1%	Mandarins	0,1%	Oranges	0,0%	Grapefruits		0,2%
	0,2%	RO general	0,09	0,1%	Oranges	0,0%	Grapefruits	0,0%	Grapefruits		0,2%
	0,2%	FR infant	0,09	0,1%	Oranges	0,1%	Mandarins	0,0%	Grapefruits		0,2%
	0,1%	DK child	0,06	0,1%	Oranges	0,0%	Mandarins	0,0%	Grapefruits		0,1%
	0,1%	DK adult	0,06	0,1%	Oranges	0,0%	Mandarins	0,0%	Grapefruits		0,1%
	0,1%	PL general	0,03	0,0%	Lemons	0,0%	Mandarins	0,0%	Oranges		0,1%
	0,0%	LT adult	0,02	0,0%	Oranges	0,0%	Mandarins	0,0%	Lemons		0,0%
	0,0%	IE child	0,02	0,0%	Oranges	0,0%	Grapefruits	0,0%	Lemons		0,0%

Conclusion:

The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.

The long-term intake of residues of PHQ is unlikely to present a public health concern.

2.7.10 Proposed MRLs and compliance with existing MRLs

The EU MRLs for OPP are currently set under Regulation (EC) 2018/78.

The MRL for the representative crop group citrus fruit is supported by the data presented in this document, and exceedance of the current MRL is not expected.

The MRL for OPP in citrus fruit is shown in the table below:

Table 2.7.10-1: Current and calculated EU MRLs for OPP in citrus fruit

Commodity	Current EU MRL[(a)] (mg/kg)	Calculated EU MRL(mg/kg)
Citrus fruit (0110000)	10.0	4.0

(a) Monitoring residue definition 2-phenylphenol (sum of 2-phenylphenol and its salts and conjugates, expressed as 2-phenylphenol). Existing MRLs for citrus fruits are based in internationally recommended CXLs established for 2-phenylphenol.

EU MRLs for OPP in products of animal origin are currently set at the LOQ of 0.01 mg/kg by default. Since no exceedance to the current MRLs is expected from the intended use of OPP on citrus, no change to the current MRLs is proposed.

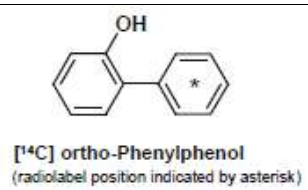
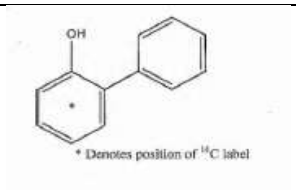
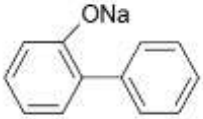
2.7.11 Proposed import tolerances and compliance with existing import tolerances

Not relevant.

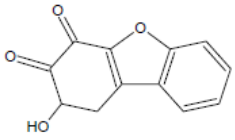
2.8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

The fate and behaviour studies for 2-phenylphenol and incl. sodium salt orthophenyl phenol were conducted with ^{14}C-phenyl- radiolabelled OPP and ^{14}C-phenol- radiolabelled OPP.

Nomenclature:

2-phenylphenol (OPP) ISO common name: o-phenylphenol Synonym: 2-hydroxybiphenyl, orthophenyl phenol Molecular formula: $C_{12}H_{10}O$ Molecular mass: 170.2 g/mol CAS Number: 90-43-7	 **^{14}C-phenyl-labelled OPP**	 **^{14}C-phenol-labelled OPP**
sodium biphenyl-2-olate (SOPP) Common name: sodium salt orthophenyl phenol Synonym: Na-OPP, SOPP, Preventol ON extra Molecular formula: $C_{12}H_9NaO$ Molar mass: 192.19 g/ml CAS: 132-27-4		

Relevant environmental metabolites

Compound	Structural formula	Compartment / study in which compound was detected
Diketohydroxy-compound (2-Hydroxy-1,2-dihydrodibenzo*[b,d]*furan-3,4-dione)		Phototransformation in Water

Sodium salt orthophenyl phenol (SOPP) is fully registered under REACH as a substance manufactured and/or imported in the European Economic Area in 10 - 100 tonnes per year.
SOPP and its conjugated acid ortho-Phenylphenol (OPP) exist in aqueous solutions in a pH dependant equilibrium.

Under neutral and acidic conditions, the equilibrium shown in Figure 2.8-2 is shifted to the side of protonated OPP. At high pH values the anionic form is the predominant molecule (pKa = 9.5).

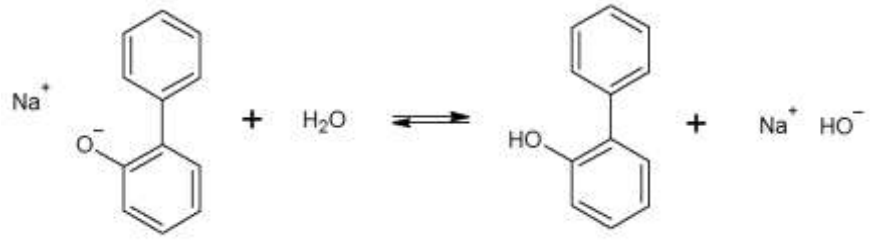


Figure 2.8- 2: Equilibrium of SOPP and OPP (pKa = 9.5) in aqueous solution

Under environmentally relevant pH conditions sodium 2-biphenylate will dissociate on contact with water forming hydrolysed Na^+ and OH^- ions and the protonated 2-phenylphenol (OPP). Consequently dissociation of sodium 2-biphenylate to 2-phenylphenol is also relevant for toxicity testing. 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. The SOPP and the OPP are expected to have a similar environmental fate and ecotoxicity profile due to the comparable physico-chemical properties of both substances. The SOPP and the OPP are characterised by a low vapour pressure (1.2 and 0.474 Pa at 20 °C respectively) and low adsorption potential (log Koc <3). The water solubility of Sodium 2-biphenylate is > 1000 g/L at pH 13.6 and 20 °C. However, as indicated by the measured dissociation constant for the substance (pKa(2-phenylphenol) = 9.5) Sodium 2-biphenylate will dissociate forming 2-phenylphenol under environmental relevant pH (pH 5 - 9). The measured water solubility of 2-phenylphenol ranged from 0.53 – 0.64 mg/L (pH 5-9 at 20°C).

2.8.1 Summary of fate and behaviour in soil

2.8.1.1 Route of degradation in soil

2.8.1.1.1 Aerobic degradation in soil

The route of degradation of OPP was investigated by Fliege R., (2005). Radiolabeled OPP was applied to sandy loam soil and incubated under aerobic conditions in the dark at 20°C (19.0 – 20.8°C) for 127 days. The application rate was 500 g a.s./ha, equivalent to 0.648 mg a.s./kg soil dw.
The percentage of ^{14}C-OPP decreased from 101.6% at time 0 to 0.6% of applied radioactivity at 127 days.
No relevant amounts of transformation product were found in extracts at any time point. The largest individual unknown component accounted for 1.6% of applied radioactivity. The total sum of unidentified components ranged from 0.9% to 10% of applied radioactivity and consisted of many minor components of less than 1% applied radioactivity.
[^{14}C] carbon dioxide and non-extractable soil residues were identified as final sinks of the applied radiocarbon. The majority of applied radioactivity was detected in the non-extractable residues and was associated with soil humin and humic acid fractions.
Non-extractable [^{14}C] residues increased from 3.6 % of applied at 0 hours, to reach a maximum of 85.2 % at day 2. Upon further incubation, a subsequent decline was observed, dropping to 77.4 % at day 127.
CO_2 accounted for a maximum of 9.6% of applied radioactivity and VOCs were not detected above 0.1% of applied radioactivity.

2.8.1.1.2 Anaerobic degradation in soil

The anaerobic soil degradation of OPP was not investigated based on the Commission Regulation (EU) 283/2013 where it is states that these studies shall be submitted unless the applicant shows that exposure of the plant protection products containing the active substance to anaerobic conditions in unlikely to occur for the intend uses. In this case, the proposed representative use of OPP in the dossier is an indoor application to post-harvest citrus fruit. The used application solution is treated as chemical waste and therefore, anaerobic conditions are not expected to occur.

2.8.1.1.3 Photodegradation in soil

The phototransformation of [^{14}C]-OPP was investigated on a sandy clay loam soil under aerobic conditions by Schaefer E., et al., 2018. Samples were irradiated by xenon arc lamp at 25°C for 15 days, equivalent to 29.2 days of natural summer sunlight at 30 to 50 °N. [^{14}C]-OPP irradiated on the soil surface mostly transformed to non-extractable residues. Non-extractable residues increased from 0.8% AR to 66.0% after 15 days of irradiation and 86.6% after 15 days in the dark. Non-extractable residues were further characterized. The majority of applied radioactivity was associated with the humin fraction. Slightly more CO_2 was evolved in irradiated samples compared to dark samples, 8.2% AR after 15 days irradiation and 4.0% AR after 15 days in the dark. Three unknown metabolites were identified, but the sum of these peaks accounted for less than 7.5% AR at any timepoint. The single first order half-life of OPP was 0.13 days, corresponding to 0.253 solar days (light) and 0.16 days, corresponding to 0.319 solar days (dark).

2.8.1.1.4 Overall route of degradation in soil

Concluded from the observed metabolic profile, degradation of ortho-phenylphenol in soil starts with an extensive coupling to the soil matrix within hours, with no pronounced formation of soluble intermediates. Although not extractable, the immobilized residues are moderately mineralized, indicating their participation in soil carbon turnover and breakdown of the radiolabel-containing phenylphenol core structure.
The observed behavior is in-line with literature information on rapid and irreversible soil binding of similar phenolic type compounds. Such effect has been attributed to oxidative coupling reactions, which may be both biologically mediated, or abiotic surface-catalyzed processes.

The major aerobic metabolic pathway in soil is presented in the figure below:

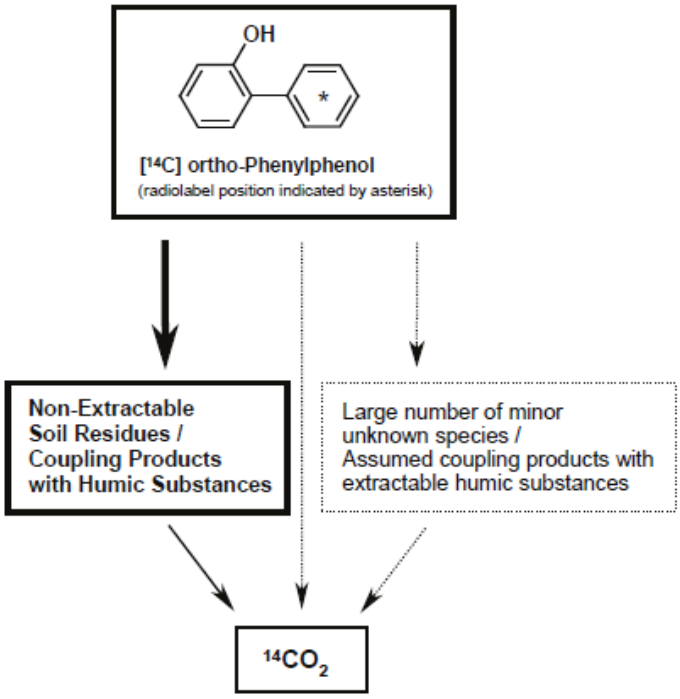


Figure 2.8.1.1.4-1: Aerobic soil degradation pathway

2.8.1.2 Rate of degradation in soil

2.8.1.2.1 Laboratory conditions

The rate of degradation of OPP was investigated by Fliege R., (2005). Persistence and modelling endpoints for 2-phenylphenol generated from laboratory aerobic soil have been kinetically re-evaluated according to FOCUS Kinetics guidance (2006, 2011, and 2014). A Q10 value of 2.58 was used for normalisation (EFSA, 2007).

Table 2.8.1.2-1: Rate of degradation in soil (aerobic) laboratory studies active substance. Modelling endpoint

Parent	Dark aerobic conditions. Persistence and modelling endpoints.						
Soil type	OC	pH[a)]	t. °C / % MWHC	DT_{50} /DT_{90} (d)	DT_{50} (d) 20 °C pF2/10kPa[b)]	t.(χ^2)	Method of calculation
Sandy clay loam	2.5%	6.0	20/50	0.10/0.46	0.14[c) d)]	1.349	FOMC
Geometric mean (if not pH dependent)					0.14		
pH dependence,					n/a		

[a)] Measured in calcium chloride solution
[b)] Normalised using a Q10 of 2.58 and Walker equation coefficient of 0.7
[c)] Moisture correction factor > 1
[d)] DT50 = DT90/3.32

2.8.1.2.2 Field dissipation studies

Not relevant.

2.8.1.2.3 Soil accumulation studies

Not relevant.

2.8.1.2.4 Assessment of Persistence (P) in soil

The assessment of P criterion was made selecting best-fit kinetics as recommended by SANCO GD together with a temperature of 20 ºC. No normalization to moisture conditions was considered. Model input dataset was the residual ortho-phenylphenol found in sum of 'ambient' plus 'aggressive' extracts at the sampling intervals 0-24 hours (time of decline to <10 % AR).
According to SANCO Working Document, unextractable residues were excluded from further assessment. They can be considered degradation loss, not bioavailable and therefore unable to exert toxicity
Trigger (persistence) DT_{50} and DT_{90} values at 20 ºC and 50% MWHC were calculated to be 2.4 and 11.1 hours respectively using FOMC modelling.
The data relevant for deriving persistence endpoints are shown in the table below:

Table 2.8.1.2.4-1: Degradation rates

Parent	Aerobic condition					
Soil Type	pH [0.01 M $CaCl_2$]	T(ºC)/ MWHC (%)	DT_{50} /DT_{90} (h) at 20ºC	DT_{50} /DT_{90} (d) at 20ºC	Error level test χ^2-test	Method of calculation
Sandy loam soil	6.0	20ºC/50 %MWHC	2.65/8.81	0.11/0.37	4.77	SFO
			2.39/11.09	**0.10/0.46**	**1.37**	**FOMC**
			2.43/11.27	0.10/0.47	1.44	DFOP

Since the DT_{50} value of OPP derived from the laboratory study at 20 °C does not exceed 60 days and DT_{90} does not exceed 200 days, nor soil dissipation neither a soil accumulation testing with OPP would be required.
Based on the study results, ortho-phenylphenol may be expected to not persist in a viable soil environment.

Overall, 2-Phenylphenol does not fulfill the persistence criterion in soil set out in points 3.7.1.1 (POP criteria), 3.7.2.1 (PBT criteria), 3.7.3.1 (vPvB criteria) of annex II of the regulation 1107/200

2.8.1.3 Mobility in soil

2.8.1.3.1 Adsorption/Desorption studies

The adsorption/desorption of OPP in four soils was determined by Oddy A., 2005 in accordance to the OECD Guideline for Testing of Chemicals No. 106.
The results of the preliminary stages of the study strongly suggested that binding of the 2-phenylphenol was not a simple equilibrium process and therefore not readily measured by the batch equilibrium methodology. The adsorption to soil was shown to be largely irreversible since the adsorbed radioactivity could not be extracted even using harsh solvents.
In order to comply with the requirements of the guideline being followed it was, therefore, considered necessary to limit the adsorption and desorption times to restrict the degree of irreversible binding and attempt to investigate the characteristics of the reversible, equilibrium process.
The Koc values determined therefore represent a very worst case for adsorption and not a realistic description of mobility under conditions of the field.
Under the latter conditions, 2-phenylphenol has to be considered as immobile due to the strong and irreversible binding to soil particles.

Table 2.8.1.3.1-1: Soil adsorption of 2-phenylphenol

Parent							
Soil Type	OC %	Soil pH[a)]	K_d (mL/g)	K_{doc} (mL/g)	K_F (mL/g)	K_{Foc} (mL/g)	1/n
Clay loam	1.9	7.1	n/r	n/r	7.47	393	0.809
Sandy loam	2.4	7.3	n/r	n/r	8.53	355	0.821
Sandy silt loam	3.0	5.2	n/r	n/r	11.66	389	0.870
Clay loam	2.8	6.2	n/r	n/r	7.04	252	0.784
Geometric mean (if not pH dependent)*					8.68	**347**	
Arithmetic mean (if not pH dependent)							**0.820**
pH dependence,			No				

2.8.2 Summary of fate and behaviour in water and sediment [equivalent to section 11.1 of the CLH report template]

Method	Results*	Key or Supportivestudy[1]	Remarks	Reference
Ready biodegradability OECD 301B	71-76% degradation after 28 days	The study is considered as supplementary information.	Readily biodegradable	Gonsior, S., Tryska, T. (1997)
Ready biodegradability Modified OECD 301E	88% degradation after 3 days. 100% degradation after 14 days	The study is considered acceptable.	Readily biodegradable	Kanne, R. (1989a)
Ready biodegradability Modified OECD 301B	89% degradation after 3 days. 100% degradation after 6 days	The study is considered acceptable.	Readily biodegradable	Kanne, R. (1989b)
Ready biodegradability EEC respirometry method: DG X1/283/82. Similar to OECD 301C.	>60% degradation after 10 days. 96% degradation after 28 days	This study is considered as supplementary information.	Readily biodegradable	Painter, A., King, E. (1984)
Aerobic aquatic metabolism in water/sediment systems. Not guidelineindicated	DT_{50}< 14 days	The study is considered as supplementary information.	Not persistent in a water/sediment system	Bruns, E. (2005)
Inherent biodegradability Zahn Wellens guideline. Similar to	100% degradation after 10 days	Not GLP. This study is considered as supplementary information.	Inherently biodegradable	Wellens, H. (1990)

Method	Results*	Key or Supportivestudy[1]	Remarks	Reference
OECD 302B				
Hydrolysis OECD 111	DT_{50}> 1 year at 50°C, pH 4, 7 and 9.	The study is considered acceptable.	Hydrolytically stable.	Reusche, W. (1990)
Photolysis in water US-EPA Series 161-2; Canadian PMRA, Daco No. 8.2.3.3.2; SETAC Section 10.	DT_{50} of 0.3 days under xenon lamps (experimental). DT_{50} of 1.7 and 2.6 solar summer days for Phoenix, AZ, USA and for Athens, Greece, respectively (calculated)	The study is considered acceptable.	Photolytically unstable	Heinemann, O (2005).
Photolysis in water (as described in literature: Environ. Sci. Technol., 32, pp. 1319-1328, 1998).	DT_{50} = 5.3 (pure water) and 4.6 days (contaminated lake water)	The study is considered as supplementary information.	Photolytically unstable	Wick and Gschwend (1998)

Based on the results of four studies of ready biodegradability following protocols like OECD 301B, 301E and 301C, *ortho*-Phenylphenol is considered to be readily biodegradable. The observed rapid degradation met the criteria of >60% degradation within a 10-day window in several of these tests. Therefore *ortho*-Phenylphenol can also be considered as rapidly degradable.

The inherently biodegradability of 161 substances (all benzene derivatives) was determined using the Zahn Wellens test resulting also inherently biodegradable, with 100% biodegradation in 10 days. However, for classification purposes this cannot be interpreted as evidence of rapid degradation, only the potential for ultimate biodegradation can be assumed.

OPP was determined to be hydrolytically stable in the study of Reusche W. 1990, degrading by less than 10% after 5 days at 50°C in pH4, pH7 and pH9 buffers.

OPP degraded rapidly in the aqueous phototransformation test by Heinemann O., 2005. The concentration of OPP decreased from 99.9% applied radioactivity on day 0 to 0.6% AR on day 7..The DT50 of OPP was 0.3 days, equivalent to 1.7 solar summer days in Phoenix, Arizona (33.3°N) or 2.6 summer days in Athens, Greece (38.0°N).

In another laboratory study (Wick L., Gschwend P., 1998) the direct photodegradation rate of 2-phenylphenol observed in pure water under summer sunlight was 0.13 d^{-1} (DT50 = 5.3 days) and had a quantum yield of 0.044 (s = ±0.001, n = 3). In lake water, the direct-plus-indirect photolysis rate constant was of 0.15 d^{-1}.

A study of the fate of OPP in a water/sediment system according to OECD 308 was not carried out. Instead, information on the degradation of 2-phenylphenol under aerobic aquatic conditions is available. Bruns E., 2005 developed screening experiments concerning the behaviour of OrthoPhenylphenol (OPP) in a “Water-Sediment System” as part of a study to determine the toxicity of OPP to chironomids,where it was observed that OPP is not stable in the aquatic compartment.

Two range finding tests were carried out in accordance with guidelines OECD 218 and OECD 219Whether OPP was bound irreversible to sediment particles or was biodegraded cannot be clarified on the basis of the available set of data.
Only dissipation from the water columns could be estimated from the limited number of analytical measurements (3-4 sampling points) of these experiments.
The exact DT50 values could not be estimated but in the tests (total recoveries from the spiked water, spiked sediment and definitive test), the amount of OPP detectable via chemical analysis was reduced by 50% or more within 14 days (DT50 <14 d). However, it was not confirmed that degradation was due to disipation or ultimate biodegradation.

The methodology of these experiments significantly deviated from the appropriate methodology of a water-sediment study; however, since OPP is demonstrated to be ready-biodegradable and considering the results of these screening data, it is not expected that the criteria for persistence (DT50 greater than 60 days for POP or 40 days for PBT in case of fresh water and the corresponding values for sediment) is met.

A study to determine the aerobic mineralization of OPP in surface water was not provided. In accordance with Regulation (EU) 283/2013, studies on aerobic mineralization in surface water shall be provided unless the applicant shows that contamination of open water (freshwater, estuarine and marine) will not occur. The proposed use of OPP in this active substance renewal submission is a post-harvest fungicidal treatment of citrus fruits. OPP is applied in a closed system, indoors. The waste water from cleaning processes should be treated as chemical waste in accordance with local legislation.

Overall, 2-Phenylphenol does not fulfill with the persistence criterion in aquatic systems set out in points 3.7.1.1 (POP criteria), 3.7.2.1 (PBT criteria), 3.7.3.1 (vPvB criteria) and vPvB substances.

Simulation studies give an indication of the potential of *ortho*-Phenylphenol as rapidly degradable. The most relevant data showing the rapid biodegradability of *ortho*-Phenylphenol were the studies of ready biodegradability. This supports that *ortho*-Phenylphenol can be considered a rapidly degradable substance.

2.8.2.1 Rapid degradability of organic substances

2.8.2.1.1 Ready biodegradability

The ratio between vapour pressure and water solubility resulted in a Henry`s Law constant of 0.14 x $10^{-3}$ $Pa \times m^3 \times mol^{-1}$ at 20 °C and pH 7. Vapour pressure and calculated Henry`s Law constant indicate that Biphenyl-2-ol has a low potential for volatilisation. Therefore, the results of the following ready biodegradability tests are not influence by the volatility of the substance.

Gonsior, S., Tryska, T. (1997).
OPP was investigated for its ready biodegradability in a CO_2-Evolution Test (OECD guideline 301B), as a modification, the test substance was applied in lower concentrations as those stipulated in the guideline (0.2 and 1 mg/L). The study was conducted under GLP.

Reaction mixtures amended with ^{14}C-OPP were sampled on days 0, 7 and 28 d to measure the amount of ^{14}C-OPP and total radioactivity in solution. After addition of acetonitrile, the samples were shaken and filtered. The biological oxygen demand (BOD) of each test bottle was measured for 28 days, values at day 7, 14, 21 and 28 were reported. Dissolved organic carbon (DOC) was determined at the end of the test. The concentration of the test substance was determined at day 28 by HPLC after dissolving the whole content of each bottle in acetonitrile. At day 0 and day 28 the pH value in each test bottle was measured.

Extensive biodegradation of ortho-Phenylphenol was observed. By day 11, 62.5-67.7% of the radioactivity added to the reaction mixtures was mineralized to $^{14}CO_2$. This rate of mineralization met the guideline criteria of 60% theoretical CO_2 production obtained within a 10-day window in the 28-day test. After 28 days biodegradation rates of 70.8-75.7% were measured.
Since little $^{14}CO_2$ was measured in the abiotic controls (<1%), the mineralization of [14C]-OPP to $^{14}CO_2$ was determined to be biologically mediated

With the data provided it is not possible to know whether the tested concentrations of OPP are in the range established in the OECD 301B (10 – 20 mg DOC or TOC/L). In order to validate the study, the information about the content of inorganic carbon (IC) of the test substance suspension in the mineral medium at the beginning of the test and the total CO_2 evolution in the inoculum blank at the end of the test is considered essential. The study is considered as supplementary information

Kanne (1989a)
OPP was investigated for its ready biodegradability in a Modified OECD Screening Test (OECD guideline 301E). The study was not conducted under GLP.

Degradation was followed by DOC determinations at different intervals (day 0 (hour 0), day 1 (24 h), and on day 2, 3, 4, 8, 9, 11, 14, 15 and 16). The concentration tested was 23.077 mg a.s./L (19 mg DOC /L).
The exposure period was 16 days, since the test substance was completely degraded already after 14 days. After 3 days, 88% of the applied o-Phenylphenol was degraded in the OECD Screening Test. The observed rapid degradation met the guideline criteria of 60% theoretical CO2 production obtained within a 10-day window in the 28-day test. After 14 days biodegradation of the test substance was complete (100%). The reference substance aniline showed a degradation of 94% after 3 days.

According to the results of the test, o-Phenylphenol can be classified as readily biodegradable.

The study is considered acceptable.

Kanne (1989b).
Another test was performed according to the modified OECD Screening Test (OECD guideline 301E) but usingRhine river water instead of deionised water. The inoculation with sludge was therefore not carried out. No abiotic control (with sterilizing agent), and no toxicity controls were investigated. The study was not conducted under GLP.

Reaction mixtures were sampled on days 0, 1, 2, 3 and 6 to measure the amount of dissolved organic carbon (DOC) in the test solutions (sample quantity 20 mL).
After 3 days 89% of applied test substance was degraded, after 6 days the degradation rate was 100%. The reference substance aniline showed a degradation of 33 and 89% after 3 and 6 days, respectively. According to the results of the test, o-Phenylphenol can be classified as readily biodegradable.

The study is considered acceptable.

Painter, A., King, E. (1984).
This is a ring test programme to extend the experience of the use of the EEC respirometric method in the 12 EEC countries, which participated in a previous ring-test (1982).
The respirometric method used for this study is similar to the Modified MITI I method (OECD Guideline 301 C) but differs in that it employs an activated sludge inoculums and a more buffered medium containing an increased concentration of ammonium salts. 14 chemicals were tested.

ortho-Phenylphenol showed > 60% biodegradation after 10 days, and 96% degradation after 28 days. OPP can be classified as readily biodegradable.

In eight of the ten test laboratories the guideline criteria of 60% theoretical CO_2 production obtained within a 10-day window (td + 10 d) was fulfilled during the 28-day test period. All ten participants reported >60% ThOD at day 28. These results are confirmed by the detected values for DOC removal; this parameter was investigated in seven of the ten tests. The mean value for DOC removal after 28 days was 96% (minimum 89%, maximum 100%). According to the results of the test, o-Phenylphenol can be classified as readily biodegradable.

This information is considered as supplementary information.

2.8.2.1.2 BOD5/COD

Not data provided.

2.8.2.2 Other convincing scientific evidence

2.8.2.2.1 Aquatic simulation tests

Water/sediment degradation

Bruns, E. (2005).
An ecotoxicological study towards sediment dwellers was performed by Egeler and Gilberg (2005) (see point 2.9.2.3.4) according to the OECD guideline 219, and analytical screening data have been generated on the dissipation of OPP in a water sediment system. Bruns (2005) summarised its results (Egeler and Gilberg, 2005) in order to investigate on the degradation of OPP in aquatic systems.

The dissipation of OPP in a water-sediment system was monitored. Two range finding tests using spiked sediment (chemical analysis at nominal concentrations of 20 and 500 mg/kg) and spiked water (chemical analysis at nominal concentrations of 4 and 100 mg/L) and the respective definitive test using water spiking (analytically investigated nominal concentrations of 1 and 4 mg/L), were used to estimate the dissipation time (DT_{50}) of OPP. The concentrations of OPP in surface water, pore water and sediment of the spiked water, spiked sediment samples and samples from the definitve test were determined at 0, 7 or 8 and 28 days.

The concentration of OPP in surface water and pore water rapidly decreased, whilst the concentration of OPP in sediment initially increased, then decreased in 28 days. Whether OPP was bound irreversible to sediment particles or was biodegraded cannot be clarified on the bases of the available set of data.

It was not possible to determine exact DT_{50} values of OPP by non-linear regression. The DT_{50} of OPP seems to be the longest in the sediment fraction (compared to overlaying water and pore fraction). In all three tests, the amount of OPP detectable via chemical analysis was reduced by 50% or more within 14 days (DT_{50}< 14 days). Whether OPP was bound irreversible to sediment particles or wasbiodegraded cannot be clarified on the basis of the available set of screening data.

This calculation is considered as supplementary information:

- The design of the test is not intended for studying the biodegradation route of OPP. Whether OPP has been bound irreversible to sediment particles or has been biodegraded cannot be clarified on the basis of the screening data.

- The generated data were only intended for screening purposes and the number of analytical measurements was limited (3-4 sampling points).

- The water and sediment are not sampled from natural SW systems but they are artificially prepared in order to support sediment dwelling organisms. These characteristics may influence in the rate of dissipation and/or degradation of the OPP.

Based on the results obtained from the analytical monitoring of an OPP toxicity test towards sediment dwellers, OPP seems to be not persistent in the water-sediment system.

2.8.2.2.2 Field investigations and monitoring data (if relevant for C&L)

Refer to 2.8.4 Summary of monitoring data.

2.8.2.2.3 Inherent and enhanced ready biodegradability tests

Wellens, H. (1990).
The biodegradability of 161 substances (all benzene derivatives), was determined using the ZahnWellens test. 2-phenylphenol (OPP) was one of the tested substances (Wellens H., 1990). OPP degraded by 63% after 5 days and by 100% after 10 days. OPP is readily biodegradable in the Zahn-Wellens test because more than 60% biodegradation was observed within a 10-day window.

The study was not conducted under GLP and it is considered as supplementary information.

2.8.2.2.4 Soil and sediment degradation data

Refer to point 2.8.2 Overall summary

2.8.2.2.5 Hydrolysis

Reusche, W. (1990).
The hydrolysis of OPP was studied in sterile aqueous buffered solutions at pH 4 (phthalate buffer), pH 7 (phosphate buffer) and pH 9 (borate buffer) according to OECD guideline 111 and GLP. Deviations observed did not affect the outcome of the results although only one vessel was investigated at each sampling time for each pH level. The concentrations of OPP were measured via HPLC-UV.

In the preliminary test at 50 ºC, a percentage of OPP of less than 10% was hydrolysed during 5 days. Considering the hydrolytic stability determined under stringent temperature conditions and at different pH values it is not expected that hydrolytic processes will contribute to the degradation of OPP in the aquatic systems (estimated DT50 > 1 year).

According to OECD guideline 111, the test substance is considered to be hydrolytically stable. This result corresponds to a half-life of far more than one year for all temperature and pH values investigated.

The study is considered acceptable.

2.8.2.2.6 Photochemical degradation

Heinemann, O (2005).
A study on photolysis of OPP in water was conducted following several current standard methods (US-EPA Series 161-2, Canadian DACO 8.2.3.3.2 and SETAC Section 10 guidelines). The study was conducted under GLP.

[phenyl-UL-^{14}C]-2-phenylphenol was incubated in a sterile aqueous buffer solution (pH 7) at a concentration of 1 mg a.s./L (total incubation time: up to 7 days at 25 °C). Duplicates were either kept in the dark or exposed to a xenon lamp.

The degradation of OPP and formation of transformation products was only observed in the irradiated samples. No degradation of OPP was observed in the dark controls. The recovery of applied radioactivity was 94.5% at the end of the test, thereof 23.7% was CO2 and 0.4% volatiles, and the rest of the radioactivity was found in the solution, i.e., as OPP and transformation products. Diketohydroxy-compound (maximum 13.6% AR) and benzoic acid (maximum 7.9% AR) were identified as the major transformation products, other 3 unidentified compounds were found to have a maximum between 1% and 10% of the AR. All transformation products occurred transiently and decreased to amounts of < 5% AR after 7 days (end of the study). A small portion of OPP was found in the volatile fraction. Degradation also took place by mineralization.

OPP is rapidly photodegraded in sterile aqueous 0.01 M phosphate buffer (experimental DT50 = 0.3 days) and photolysis plays an important role for the degradation of OPP in the aquatic compartment. Although OPP's λmax were reported to be at 243 and 283 nm, the termination of absorption was observed above 290 nm.

Based on the experimental DT50 the predicted environmental DT50 is calculated to be 1.7 solar summer days at Phoenix (USA) or 2.6 summer days at Athens (Greece). OPP is not likely to be photolytically stable in aqueous medium.

The major metabolite was diketohydroxy-compound. The DT50 of diketohydroxy-compound was 1.3 days, equivalent to 7.2 solar summer days in Phoenix, Arizona or 11.1 summer days in Athens, Greece.

The study is considered valid.

Wick, L.Y. and Gschwend, P.M. (1998).
The photodegradation rate of OPP in pure and lake water was determined following a method described in the literature. Measurements were performed under natural sun light conditions for pure water. The quantum yield was determined in pure water as well.

Investigations were done in a small lake (Halls Brook Holding Area, Woburn, MA, USA) receiving discharges contaminated with o-Phenylphenol from a superfund site. In year-around studies chemicals concentrations were measured and laboratory experiments regarding rates of specific processes were done.

To asses direct photolysis rates, pure water was used. Lake water samples were taken from about 10 cm below the

surface, filtered and poisoned with HgCl2 to determine direct plus indirect photolysis. Water samples containing OPP were made 2 x 10^{-5} M, placed into 1.3 cm (outer diameter) x 10 cm quartz tubes, stopped and irradiated in sunlight during July and August 1996. Over time, samples were sacrificed to be analysed and quantified by reverse-phase HPLC.

The direct photodegradation rate of OPP observed in pure water, oxygen-containing water under summer sunlight was 0.13 d^{-1} (DT50 = 5.3 days) and had quantum yield of 0.044 (s = ± 0.001, n = 3).
In lake water, OPP showed a direct-plus-indirect photolysis rate constant of 0.15 d^{-1} (DT50 = 4.6 days). Taking into account light attenuation in the lake water (alpha (300 nm) = 12 m-1) direct photolysis would account for about 75% of light-induced removal; in situ photochemical degradation rates would be about 100 times slower than observed in the quartz tubes.

The study is considered as additional information.

2.8.2.2.7 Other / Weight of evidence

Refer to 2.8.4 Summary of monitoring data.

2.8.3 Summary of fate and behaviour in air

The vapour pressure of 2-phenylphenol (0.474 Pa at 20°C) indicates that losses due to volatilization would not be excluded. However, the proposed use of OPP in this active substance renewal submission is a post-harvest fungicidal treatment of citrus fruits. OPP is applied in a closed system, indoors. There would be no volatilisation to the environment

Additionally, calculations using the method of Atkinson (using the software APOWIN, v.1.91) for indirect photo oxidation in the atmosphere through reaction with hydroxyl radicals resulted in an atmospheric half-life estimated at 0.59 days (assuming an atmospheric hydroxyl radical concentration of $0.5x10^6$ OH-radicals cm^3 as average for 24 hours a day). This half-life indicates that the proportion of 2-phenylphenol which is volatilized is unlikely to be subject to long-range atmospheric transport.

Local and global effects of an active substance are to be investigated for substances that are applied in high amounts. OPP is applied indoors and is not applied in high amounts.
Global warming potential, ozone depleting potential, photochemical ozone creation potential and accumulation in the troposphere are all unlikely to occur following use of OPP according to good agricultural practice. The DT_{50} of OPP in air (tropospheric DT_{50} = 0.59 days) is too short to enable accumulation.
The acidification potential of OPP is low as use of the substance does not generate acidifying gases like sulphur dioxide or nitrous oxides in a free form.
The eutrophication potential of OPP is low as use of the substance does not generate ammonia or phosphorous compounds which cause eutrophication by increasing the available nutrients for the relevant aquatic organisms.

Based on the available data the RMS concludes that 2-Phenylphenol does not fulfill the POP-criterion for potential for long-range environmental transport as stated in Annex II to Reg (EC) 1107/2009.

2.8.3.1 Hazardous to the ozone layer

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone layer

Global warming potential, ozone depleting potential, photochemical ozone creation potential and accumulation in the troposphere are all unlikely to occur following use of OPP according to good agricultural practice. The DT_{50} of OPP in air (tropospheric DT_{50} = 0.59 days) is too short to enable accumulation.

There are no data provided regarding the hazard of *ortho*-Phenylphenol to the ozone layer, the Ozone Depleting Potential (ODP) of *ortho*-Phenylphenol has not been measured.

2.8.3.1.2 Comparison with the CLP criteria

A substance is considered hazardous to the ozone layer if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer.
Any substances having an ODP of greater than or equal to the lowest ODP (i.e., 0.005) of the substances currently listed in Annex I to Regulation EC No 1005/2009 should be classified as hazardous to the ozone layer (category 1).
Although no specific data have been provided for this hazard, considering the chemical structure and other available information on the physicochemical properties, *ortho*-Phenylphenol is not expected to be hazardous to stratospheric ozone.

2.8.3.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not classified, data lacking.

Not data submitted.

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.

2.8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

2.8.4.1 Surface water

Three papers were presented, one from 1998 (Germany), one from 2014 (Germany) and one from 2016 (Spain).
In Ternes T., et al., 1998, 2-Phenylphenol was found in the majority of the samples taken from rivers and streams in Germany.
Concentrations of 2-phenylphenol above 0.1 µg/L were found in 7 of 82 samples from municipal STP discharges. In river and streams, mainly OPP was found in concentrations comparable to STP discharges. In two of 31 samples, OPP was found above 0.1 µg/L. Elimination rates of 98 % for OPP were obtained in one STP situated near Frankfurt/Main
Jewell K., et al., 2014 included data on concentration of OPP in 2 WWTPs. Concentrations of OPP decreased from the low mg/L range before the activated sludge reactor to the low ng/L range in the WWTP effluent in both sites studied.
Peris-Vincente J., et al., 2016, proposes a micellar liquid chromatographic method to determine thiabendazole and o-phenylphenol in wastewater. The procedure was applied to the screening of TBZ and o-phenylphenol in wastewater samples from citrus packing plants, agricultural gutters, urban sewage, as well as in influent and effluent wastewater treatment plants.
The samples taken from wastewater treatment plants demonstrate the high removal efficiency of o-phenylphenol in STPs.
The samples taken from urban sewage waters detected concentrations up to 50 µg/L. In agricultural gutter, OP was not detected in any case.
The most significant data is the extremely high concentrations found from fruit packing plant with values up to 1100 µg/L. Specially, since the wastewater from cleaning processes with OPP should be treated as chemical waste and no contaminated wastewater should leave the treatment facilities.

2.8.5 Definition of the residues in the environment requiring further assessment

The residue definitions relevant for risk assessment for each environmental compartment are as follows:

Compartment	Residue definition	Major Metabolite
Soil, Groundwater	2-phenylphenol	parent
Surface water	2-phenylphenol, Diketohydroxy-compound ((2-hydroxy-1,2-dihydrodibenzo[b,d]furan3,4-dione))	parent, aqueous photolysis metabolite
Air	2-phenylphenol	parent

2.8.6 Summary of exposure calculations and product assessment

2.8.6.1 PECsoil

AGF/1-04 is applied in a closed system, indoors. The waste water from cleaning processes are treated as chemical waste in accordance with local legislation. There will be no exposure to soil.
This is in accordance with Regulation EU 1107/2009, which defines post-harvest treatment as "treatment of plants or plant products after harvest in an isolated space where no run-off is possible, for example in a warehouse".
With consideration of the points above, PECsoil values have not been calculated.

2.8.6.2 PECgw

AGF/1-04 is applied in a closed system, indoors. The waste water from cleaning processes are treated as chemical waste in accordance with local legislation. There will be no exposure to soil and no risk to groundwater. Therefore, PECGW values have not been calculated.

2.8.6.3 PECsw and PECsed

AGF/1-04 is applied in a closed system, indoors. There will be no exposure to surface water via drift, run-off or drainage. This is in accordance with Regulation EU 1107/2009, which defines post-harvest treatment as "treatment of plants or plant products after harvest in an isolated space where no run-off is possible, for example in a warehouse".
With consideration of the points above, PECsw and PECsed values have not been calculated using FOCUS modelling software Steps 1 and 2 version 3.2, SWASH version 3.1, PRZM version 3.3.1, MACRO version 5.5.3, TOXSWA version 3.3.1 and SWAN version 4.01.
The wastewater from cleaning processes are treated as chemical waste in accordance with local legislation. Nevertheless, to simulate potential contamination of surface waters via emission from STP, PECsw and PECsed values have been calculated from PECeffluent values, which were modelled using SimpleTreat version 3.1 and SimpleTreat version 4.0.

Table 2.8.6.3: PECeffluent and PECsw calculations for active substance OPP following cleaning of drencher equipment*

SimpleTreat version	Emission type	OPP from cleaning (g)	PECeffluent (mg/L)	Dilution to freshwater	PECsw (µg/L)
3.1	Emission from 1 cleaning operation	180	0.004048	10	0.4048
	Daily emission	15.78	0.0003549	10	0.03549
4.0	Emission from 1 cleaning operation	180	0.003865	10	0.3865
	Daily emission	15.78	0.0003388	10	0.03388

*For consistency reasons these data have not been included in the List of Endpoints.

2.8.6.4 PECair

The DT50 of OPP in air is estimated as 0.59 days. The vapour pressure of OPP does trigger the requirement for further data on transport via air.
The triggers are 10-5 Pa from plants and 10-4 Pa from soil, in accordance with Regulation (EU) 283/2013. However, the proposed use of OPP in this active substance renewal submission is a post-harvest fungicidal treatment of citrus fruits. OPP is applied in a closed system, indoors. There will be no volatilisation to the environment. This is in accordance with SANCO/10553/2006 revision 2, which states "As the outdoor exposure after warehouse use depends on parameters that have not been quantified it is scientifically not justified to derive a general conclusion from these experiments. Therefore, no general recommendation on emissions from warehouses can be given here". The purpose of the experiments referred to was to determine the potential air contamination after fogging warehouses with dichlorvos.

Based on the short DT50 in air and the proposed indoor application, it is not expected that the active substance OPP be present in the air for long enough or at high enough concentrations to travel or accumulate.

2.8.6.5 Predicted environmental concentrations from other routes of exposure

No data submitted.

2.9 EFFECTS ON NON-TARGET SPECIES

2.9.1 Summary of effects on birds and other terrestrial vertebrates

Studies on the toxicity of OPP/SOPP to birds and mammals are summarised in Vol 3 CA Section 9, point B.9.1. The proposed use of OPP in this active substance renewal submission is a post-harvest fungicidal treatment of citrus fruits. OPP is applied in a closed system, indoors. There will be no exposure to terrestrial vertebrates. The results of the bird and mammal toxicity studies are provided as additional information.

Table 2.9.1-1: Summary of bird toxicity endpoints of OPP

Test type	Test species	Endpoint	Reference	Acceptability
Acute toxicity	Mallard duck	LD_{50} = > 2250 mg/kg bw	Grimes J., 1986a, KCA 8.1.1.1/01	Accepted
Short-term dietary toxicity	Bobwhite quail	LD_{50} = > 5620 ppm	Grimes J., 1986b, KCA 8.1.1.2/01	Accepted
Short-term dietary toxicity	Mallard duck	LD_{50} = > 5620 ppm	Grimes J., 1986c, KCA 8.1.1.2/01	Accepted

Table 2.9.1-2: Summary of Acute toxicity of OPP/SOPP to mammals

Method, guideline, deviations if any	Test Species	Test substance	LD_{50} (mg as/kg bw)	Reference	Acceptability
Acute oral toxicity study in rats Prior to OECD TG 401 GLP: No (prior to GLP enforcement) Deviations: Test material no characterised. Animals were not fasted; Dosing into duodenum; Necropsy: by random sample; Individual body weights not reported.	Rat	OPP	2980	Löser E., 1981	Supporting information
Acute oral toxicity study in rats Prior to OECD TG 401 GLP: No (prior to GLP enforcement) Deviations: Only brief summary written in German. Test substances not characterised; strain, sex and weight of test animals not reported; animals were not fasted; 7 days observation period; necropsy not performed.	Rat	OPP	>2500	Kimmerle G., Lorke D., 1969	Supporting information
Acute oral toxicity study in rats Prior to OECD TG 401 (1987) GLP: Not applicable. Published study Deficiences: only a brief summary. Batch of the test substance not reported; strain of animals not specified; incomplete test method description; individual body weights only recorded at the beginning of the study; necropsy not performed.	Rat	OPP	2700	Hodge H. *et al.*, 1952	Supporting information
Acute oral toxicity study in mice Not possible to check test method. GLP: Not applicable. Published study	Mouse	OPP	1200 (male) 1050 (female)	Taniguchi Y. *et al.*, 1981	Supporting information

Method, guideline, deviations if any	Test Species	Test substance	LD_{50} (mg as/kg bw)	Reference	Acceptability
Deviations: publication written in Japanese. Only abstract and results table/graphs are written in English. It is not possible to check the method. Purity of test substance not reported.					
Acute oral toxicity study in rats OECD TG 401 (1987) GLP: Yes	**Rat**	**OPP**	**2733**	**Gilbert K., Crissman J., 1994**	**Accepted**
Acute oral toxicity study in mice Not possible to check test method. GLP: Not applicable. Published study Deficiencies: publication written in Japanese. Only brief abstract and results table/graphs are written in English. It is not possible to check the method.	Mouse	OPP	3499 (male) 3152 (female)	Tayama K. *et al.*, 1983	Supporting information
Acute oral toxicity study in rats OECD TG 401 (1987) GLP: Yes	**Rat**	**SOPP**	**591 (male) 846 (female)**	**Gilbert, K.S. and Stebbins, K.E., 1994**	**Accepted**
Acute oral toxicity study in rats Prior to OECD TG 401 GLP: No (prior to GLP enforcement) Deviations: Animals were not fasted, test material not characterised, necropsy was not performed. Individual body weights were not reported.	Rat	SOPP	1720	Löser, E., 1980	Supporting information

Table 2.9.1-3: Summary of Long-term and reproductive toxicity of OPP/SOPP to mammals

Method. Guideline, deviations if any. Acceptability. Strain/Species. No of animals.	Test Species	Test substance	Test Design	NOAEL (mg as/kg bw/day)	Reference	Acceptability
Long-term study. No guideline. **Supportive only.** Wistar-derived rat. Both sexes. 25/sex and dose.	Rat	OPP	2 year, dietary	100-200	Hodge H. *et al.*, 1952 (Supplementary)	Supporting information
Combined Chronic Toxicity/carcinogenicity. OECD Guideline 453. Deviations: Age at study start older than recommended. No satellite groups. Water consumption not measured. Volume of urine not recorded.	**Rat**	**OPP**	**2 year, dietary**	**39**	**Wahle B., Christenson W., 1996 (Accepted)**	**Accepted**

Method. Guideline, deviations if any. Acceptability. Strain/Species. No of animals.	**Test Species**	**Test substance**	**Test Design**	**NOAEL (mgas/kg bw/day)**	**Reference**	**Acceptability**
Accepted. Fischer 344rats. Both sexes. 20/sex and dose in the 1-year group. 50/sex and dose in the 2-year group.						
Long-term study OECD Guideline 453. Deviations: No satellite groups. Incomplete testing and reporting. **Supportive only.** F344/DuCrj rats. Males. 20-24/ Dose group	Rat	OPP	91 week, dietary	269	Hiraga K., Fujii T., 1984 (Supplementary)	Supporting information
Dietary in, mouse. OECD Guideline 453. Deviations: No satellite groups. Haematology, clinical biochemistry and urinalyses determinations were only performed on terminal samples instead of at 3 and 6 months. More haematological parameters should have been measured. No statistical analysis on gross pathology data. **Accepted.** B6C3F1 mice. Both sexes. 60/sex and dose.	**Mouse**	**OPP**	**2 year, dietary**	**250**	**Quast J., McGurk R., 1995 (Accepted)**	**Accepted**
Long-term dermal, mouse. No guideline. **Supportive only.** Swiss CD-1 mice Both sexes. 50/sex and dose.	Mouse	OPP	2 year, dermal	55.5	National Toxicology Program, 1986 (Supplementary)	Supporting information
Two-generation, rat OECD 416. Deviations: Dose spacing and resting period before the second mating lasted longer than recommended. Cohousing period was shorter than recommended. No assessment of sexual maturation, sperm parameters, corpora lutea, and uterine implantation sites was performed. Some organ weights were not reported. **Accepted.** CD Sprague-Dawley	**Rat**	**OPP**	**2-generation reproduction**	**Parent = 35 Offspring = 125 Reproductive ≥ 457**	**Eigenberg D., 1990 (Accepted)**	**Accepted**

Method. Guideline, deviations if any. Acceptability. Strain/Species. No of animals.	**Test Species**	**Test substance**	**Test Design**	**NOAEL (mgas/kg bw/day)**	**Reference**	**Acceptability**
rats. Both sexes. At 25-35 per sex and dose group.						
Two-generation, rat OECD 416. Deviations: Same as in the previous 2-generation study by Eigenberg (1990), except dams were cohoused for appropriate amouts of time. **Accepted.** Albino CD Sprague-Dawley rats. Both sexes. 30/sex/dose.	**Rat**	**OPP**	**2-generation reproduction**	**Parent = 92 (female) Offspring = 92 (female) Reproductive ≥ 457 (female)**	**Eigenberg D., Lake S., 1995 (Accepted)**	**Accepted**
Developmental toxicity, rat No guideline. **Supportive only** Wistar strain Rat. Females. 11 to 20 / Dose group.	Rat	OPP	Developmental	Parent = 150 Offspring = 300	Kaneda M. *et al*., 1978 (Supplementary)	Supporting information
Developmental toxicity, rat No guideline. **Supportive only** SD-Rat. Females. 25 to 35 / Dose group.	Rat	OPP	Developmental	Parent = 300 Offspring = 300	John J. *et al*., 1978 (Supplementary)	Supporting information
Developmental toxicity, rabbit OECD 414. Deviations: Treatment period ended too soon. Food consumption was not recorded. Mortality was higher than 10%. **Accepted.** NZW Rabbit	**Rabbit**	**OPP**	**Developmental**	**Parent = 100 Offspring ≥ 250**	**Zablotny C. *et al*., 1991 (Accepted)**	**Accepted**
Developmental toxicity, mice No guideline. **Supportive only** JCL-ICR mice . Females. OPP: 20 to 21 / Dose group. SOPP: 20 / Dose group.	Mouse	OPP	Teratogenicity	Parent < 1450 Offspring < 1450	Ogata A. *et al.*, 1978 (Supplementary)	Supporting information
	Mouse	SOPP	Teratogenicity	Parent < 100 day Offspring < 100	Ogata A. *et al.*, 1978 (Supplementary)	Supporting information

No published data on the effects of OPP on vertebrate wildlife was found in the literature search, presented in Vol 3 CA section 9, point B.9.11.1. A study of the effects of OPP on amphibian metamorphosis was presented as part of the endocrine disruption data set. This study indicated no adverse effects of OPP on the thyroid.

2.9.2 Summary of effects on aquatic organisms [section 11.5 of the CLH report]

2.9.2.1 Bioaccumulation [equivalent to section 11.4 of the CLH report template]

Table 2.9.2.1-1: Summary of relevant information on bioaccumulation

Method	Species	Results	Key or Supportivestudy[1]	Remarks	Reference
Partition coefficient n-octanol/water OECD 117 shake-flask method and GC determination	-	LogPow (pH 6.3) = 3.18 at 22.51 ºC	The study is considered acceptable		Kausler, (1991)
Bioconcentration test Directive 67/548/EC, C.13 (1998) (equiv. OECD TG 305)	Zebra fish (*Brachidanio rerio)*	BCF = 21.7 (wet weight) (at 5 and 50 µg/L) BCF = 114 (lipid content) (at 5 µg/L) BCF = 115 (lipid content) (at 50 µg/L)	The study is considered acceptable	Negligible potential for bioaccumulation	Caspers (1999)

2.9.2.1.1 Estimated bioaccumulation

Partition coefficient n-octanol/water test.

Kausler, (1991).
Determination of logPow according to OECD 217 shake-flask method and GC determination. The study was under GLP. Although the substance is surface active the highest concentration of the test substance in water is only 0.6mg/L and therefore the effect of surface activity is negligible. Both phases were separated in a separatory funnel and centrifuged. Clear solutions were obtained.
Log Pow = 3.18 at 22.51ºC (pH = 6.3, pure water).

The study is considered acceptable.

Bioconcentration test.
Caspers, (1999)

A study was undertaken to determine the bioconcentration of OPP in fish, according to Directive 67/548/EC, C.13 (1998) (i.e., equivalent to OECD 305).

50 zebra fish (*Brachydanio rerio*) of 4 months and a mean body length between 2.5 and 3.5 cm were included in each 25L flow-through test vessel. Test substance concentrations were 0, 5.0 and 50 µg OPP/L. Fish were exposed to test substance for an uptake phase of 53 hours and concentrations were measured at 2, 6, 23, 30 and 48 hours after the start of the test. After 53 hours, fish were exposed to clean water for a depuration phase of 19 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 uptale phase and at the end of the depuration phase.

The steady state bioconcentration factor (BCF) was determined as 21.7. This indicates a negligible potential for bioaccumulation. Concentrations of OPP in water and fish rapidly reached a steady state in the uptake phase and decreased quickly during the depuration phase, thus not providing an appropriate data bases for calculation of uptake and depuration rate constants. The BCF values with consideration of the lipid content of fish were 114 at 5.0 µg OPP/L and 115 at 50 µg OPP/L.

2.9.2.2 Acute aquatic hazard [equivalent to section 11.5 of the CLH report template]

Table 2.9.2.2: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Key or Supportive study	Remarks	Reference
Acute toxicity to fish ASTM Standard E729-80 Guideline similar to OECD 203	Fathead minnow *(Pimephales promelas)* Bluegill sunfish *(Lepomis macrochirus)* Rainbow trout *(Oncorhynchus mykiss)*	OPP Purity: 99.25%	96h-LC_{50} = 5.1 mg/L (geometric mean of two 96-LC50 values: 4.7 mg/L and 5.5 mg/L) 96h-LC_{50} = 4.6 mg/L (nom) 96h-LC_{50} = 4.0 mg/L (nom)	Accepted	OECD 203 validity criteria were met	., Anonymous (1985),
Acute toxicity to fish Guideline similar to OECD 203	*Danio rerio*	OPP Purity: 99.5%	96h-LC_{50} = 4.5 mg/L (nom)	Accepted	OECD 203 validity criteria were met	Caspers, (1989a)
Acute toxicity to fish Guideline similar to OECD 203	*Onchorhynchus tshawytscha*	OPP Purity: 99.9%	96h-LC_{50} = 4.75 mg/L (nom)	Supporting information	Not GLP OECD 203 validity criteria were not met	Bradley D., (1991)

Acute toxicity to fish Guideline similar to OECD 203	Rainbow trout (*Oncorhynchus mykiss*)	SOPP Purity: 71.48%	96h-LC_{50} = 2.6 mg/L	Accepted	OECD 203 validity criteria were met	Hoberg (2006a)
Acute toxicity to fish Guideline similar to OECD 203	Bluegill sunfish (*Lepomis macrochirus*)	SOPP Purity: 71.48%	96h-LC_{50} = 5.1 mg/L	Accepted	OECD 203 validity criteria were met	Hoberg (2006b)
Acute toxicity to fish Guideline similar to OECD 203	Sheepshead minnow (*Cyprinodon variegatus*)	SOPP Purity: 71.48%	96h-LC_{50} = 5.1 mg/L	Accepted	OECD 203 validity criteria were met	Hoberg (2006c)
Acute toxicity to aquatic invertebrates Guideline: ASTM Standars E729-80 Guideline similar to OECD 202	*Daphnia magna*	OPP Purity: 99.25%	48h-EC_{50} = 2.7 mg/L (nom)	Accepted	OECD 202 validity criteria were met	Dill D., *et al.*, (1985)
Acute toxicity to aquatic invertebrates DIN 38412-11 Guideline similar to OECD 202	*Daphnia magna*	OPP Purity: not reported	48h-EC50 = 1.5 mg/L, (nom)	Supporting information	Not GLP OECD 202 validity criteria were not met	Kühn, R (1988)

Acute toxicity to aquatic invertebrates OECD 202	*Daphnia magna*	OPP Purity: 99.5%	48h-EC50 = 2.71 mg/L	Supporting information	Not GLP.	Ramos et al. (1998)
Acute toxicity to aquatic invertebrates **OCSPP 850.1035 (2016)**	**Mysid (*Americamysis bahia*)**	**SOPP** Purity: 71.48%	96h-LC_{50} = 0.32 mg/L (mm)	Accepted	validity criteria were met	Hoberg (2006d)
Acute toxicity to aquatic invertebrates OCSPP 850.1035 (2016)	Eastern oyster (*Crassostrea virginica*)	**SOPP**	EC_{50} = 3.4 mg/L (mm)	Accepted	validity criteria were met	Cafarella (2006) KCA 8.2.4.2/02
Acute toxicity to algae or other aquatic plants OECD 201	*Pseudokirchneriella subcapitata*	OPP Purity: 99.91%	72h-E_rC_{50} = 3.57 mg/L (mm)	Accepted	validity criteria were met	Hicks S., (2002)
Acute toxicity to algae or other aquatic plants German testing procedure DIN 38412 L9 (1989)	*Scenedesmus subspicatus*	OPP Purity: not reported	72h-E_rC_{50} = 0.98 mg/L (nom)	Supporting information	OECD 201 validity criteria could not be checked	Caspers, (1989c),
Acute toxicity to algae or other aquatic plants OECD 201	*Chlorella pyrenoidosa*	OPP. Purity: 98.5%	72h-ErC50 = 5.0 mg /L	Supporting information	Not GLP.	Ramos et al. (1999)

Acute toxicity to algae or other aquatic plants OECD 201	*Anabaena flos-aquae*	SOPP Purity: 71.48%	) 72h-E_rC_{50} =5.9 mg/L (mm)	Supporting information	Validity criteria were not met	Hoberg (2006e)
Acute toxicity to algae or other aquatic plants OECD 201	*Navicula pelliculosa*	SOPP Purity: 71.48%	72h-E_rC_{50} =5.7 mg/L (mm)	Supporting information	Validity criteria were not met	Hoberg (2006f)
Acute toxicity to algae or other aquatic plants Guideline similar to OECD 201	*Skeletonema costatum*	SOPP Purity: 71.48%	ErC_{50} =7.4 mg/L (mm)	Supporting information	Validity criteria were not met	Hoberg (2006g)
Acute toxicity to algae or other aquatic plants Guideline similar to OECD 221	*Lemna gibba*	SOPP	EC_{50} =6.2 mg/L (mm) (frond density) EC_{50} > 9.4 mg/L (mm) (growth rate) ErC_{50} =7.7 mg/L (mm) (frond biomass)	Accepted	OECD 221 validity criteria were met	Hoberg (2006h)

2.9.2.2.1 Acute (short-term) toxicity to fish

To assess the acute toxicity of OPP on fish three studies were available:

Dill D.*, et al,* (1985)
The acute toxicity of *ortho*-Phenylphenol was determined in a static test to the fathead minnow (*Pimephalespromelas*), bluegill sunfish (*Lepomis macrochirus*) and rainbow trout (*Oncorhychnus mykiss*) according to ASTM Standard E729-80. The test was conducted under GLP.

Fish were exposed in groups of ten per vessel for 96 hours under static conditions and mortality was recorded at 24, 48, 72 and 96 hours. Nominal concentrations were analytically confirmed at day 0 and day 4. Under the test conditions, the test substance was stable, resulting in measured values between 98% and 105% of nominal. Thus, all reported results were based on nominal concentrations of the test substance.

- Rainbow trout: groups of ten fish were exposed in dilution water for four days under static conditions to OPP at nominal concentrations of 0, 1.2, 1.5, 1.8, 2.3, 2.9, 3.6, 4.5, 5.6 and 7.0 mg a.i./L. No fish died in the controland all rainbow trout were found alive up to concentrations of 3.6 mg/L whereas fish exposed to higher concentrations (4.5, 5.6 and 7.0 mg/L) died. At dose levels of 2.9 to 4.5 mg/L, most surviving fish were immobilized. Fish exposed to 2.3 mg/L were melanized.
  Based on nominal concentrations, the 96-hour LC50 of ortho-Phenylphenol to Oncorhychnus mykiss was 4.0

mg/L.

- Fathead minnow: two static acute toxicity tests with fathead minnow were carried out:
    - groups of ten fish were exposed in dilution water for four days under static conditions to OPP at nominal concentrations of 0, 0.78, 1.3, 2.2, 3.6, 6.0, and 10.0 mg a.i./L. No fathead minnow died in the control and in the treatments with 0.78, 1.3, 2.2 and 3.6 mg a.i./L and all fish died in the highest concentrations of 6.0, and 10.0 mg a.i./L within 96 hours of exposure. Based on nominal concentrations, the 96h-LC50 of ortho-phenylphenol to fathead minnow under static conditions was 4.7 mg a.i./L.
    - groups of ten fish were exposed in dilution water for four days under static conditions to OPP at nominal concentrations of 0, 2.6, 3.3, 4.1, 5.1, 6.4, 8.0 and 10.0 mg a.i./L. No fathead minnow died in the control and in the treatments with 4.1 and 5.1 mg a.i./L (however, some fish were immobilized), while one fish died at 2.6 and 3.3 mg/L and all fish died in the highest concentrations of 6.4, 8.0 and 10.0 mg a.i./L within 96 hours of exposure. Based on nominal concentrations, the 96h-LC50 of ortho-phenylphenol to fathead minnow under static conditions was 5.5 mg a.i./L.

  The geometric mean of the two 96h-LC50 values is 5.1 mga.i./L.

- Bluegill sunfish: groups of ten fish were exposed in dilution water for four days under static conditions to OPP at nominal concentrations of 0, 3.2, 3.5, 3.9, 4.4, 4.9, 5.4 and 6.0 mg a.i./L. All fish survived up to concentrations of 3.9 mg/L. At 4.4 and 4.9 mg/L, 3 and 7 fish died, respectively. At concentrations of 5.4 and 6.0 mg/L, all bluegill died. In the dose groups of 3.5 to 4.9 mg/L, most surviving fish were swimming abnormally and some were immobilized.
  Based on nominal concentrations, the 96h-LC50 of ortho-phenylphenol to bluegill under static conditions was 4.6 mg a.i./L.

Deviations: The length of the fish used on the test was smaller (2.8 cm) that recommended in the test (5 ± 1cm). Not justification or rationale was provided about this. The acclimation period was not indicated. These deviations were not considered to have affected the outcome of the study.

The study is considered valid.

Caspers, (1989a)
An acute toxicity test of *ortho*-Phenylphenol (purity: 99.5%) to the zebra fish (*Brachydanio rerio*) was conducted following an UBA-Draft method (1984), comparable to OECD TG 203 and EC Method C.1, and in conformity with GLP.

Fish were exposed in groups of ten per vessel for 96 hours (semi-static with aeration, renewal of medium every 24 hours) to nominal concentrations of 1.1, 2.3, 4.5 and 9.0 mg./L. Mortality and abnormal swimming behaviour were recorded. Dissolved oxygen ranged from 85.5 to 92.0 %, pH from 7.4 to 8.2 and temperature from 21.6 to 22.3°C. Mean measured concentrations ranged between 84% and 98% of nominal; results were expressed based on nominal concentrations of the test substance.

No fish died in the controls or during the treatments at concentrations of 1.1 and 2.3 mg/L. At 4.5 mg a.i./L, 20% of the fish died, while at the highest concentration all fish died within 24 hours. No abnormal symptoms were observed at concentrations lower than the lowest lethal concentration (LLC) of 4.5 mg a.i./L. In this dose group fish showed indolent and lethargic swimming behaviour.

Based on nominal concentrations, the 96 hour-LC_{50} of *ortho*-Phenylphenol to zebra fish under semi-static conditions was 4.5 mg/L. The 96 hour-NOEC was 2.3 mg/L.

Deviations: Only four concentrations were tested and photoperiod was not specified. However, these deviations were not considered to affect the outcome of the study.

The study is considered valid.

Bradley D, (1991)

The acute toxicity of *ortho*-Phenylphenol was studied on the Chinook salmon (*Oncorhynchus tschawytscha*) at nominal concentrations of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 10.0 and 20.0 mg a.i./L according to Standard Methods for the Examination of Water and Waste Water (APHA 1989) and U.S. EPA (1985) under static conditions. The test was not conducted according to GLP

Fish were exposed in groups of ten per concentration for 96 hours and mortality was recorded at 24, 48, 72 and 96 hours. Dissolved oxygen ranged from 10.0 to 10.2 mg/L, pH from 5.8 to 6.5 and temperature from 15 ± 1°C.

No fish died in the controls and in the treatments with 1.0, 2.0 and 3.0 mg a.i./L, while 90% of the fish died at 4.0 mg/L, 40% at 5.0 mg/L and all fish died in the concentrations of 6.0, 10.0 and 20.0 mg/L within 24 hours of exposure.

Based on nominal concentrations, the 96 hour-LC_{50} of *ortho*-Phenylphenol to Chinook Salmon (*Oncorhynchus tschawytscha*) under static conditions was 4.75 mg/L.

Deviations: Insufficient reporting of test conditions, e.g., no information about analytical verification of test substance concentrations. Acclimation period was not specified. The validity criteria according to OECD 203 could not be checked.

The study is considered as supplementary information.

To assess the acute toxicity of SOPP on fish four studies were available:

Three studies on the acute toxicity of sodium salt 2-phenylphenol were carried out according to OPPTS Draft Guideline Number 850.1075 (Anonymous, 2006a, 2006b,2006c). The species tested were *Oncorhynchus mykiss, Cyprinodon variegatus* and *Lepomis macrochirus.* The validity criteria for OECD 203 were met and the results were considered reliable. The 96-hour LC_{50} of sodium salt orthophenyl phenol in the rainbow trout *Oncorhynchus mykiss* was 2.6 mg a.s./L. The 96-hour LC_{50} in the sheepshead minnow (*Cyprinodon variegatus*) and in the bluegill sunfish *Lepomis macrochirus* was 5.1 mg a.s./L.

Hoberg (2006a)
The acute toxicity of sodium 2-biphenylate to *Oncorhyncus mykiss* was investigated in a flow-through test at nominal substance concentrations of 1.0, 1.7, 2.9, 4.8 and 8.0 mg/L. The test solutions were replaced at a rate of 90 % every 9 hours. The test substances concentration was analytically verified at test initiation and test termination by HPLC. The mean measured concentrations were 0.68, 1.1, 2.1, 3.8 and 6.6 mg/L (68, 67, 73, 79 and 83 % of nominal). The test was conducted according to GLP.

Fish were exposed in groups of ten per concentration for 96 hours and mortality was recorded at 24, 48, 72 and 96 hours. At 96h of exposure, no fish died in the control and in the treatment with 1.1 mg a.i./L. Mortality of 10% was observed at the 0.68 mg/L treatment level. The mortality observed at this treatment level was considered incidental and unrelated to treatment because:

- 1) observed in only one replicate vessel,
- 2) the test met the acceptable control mortality criterion of < 10%, and
- 3) since no mortality was observed in the next highest treatment level (1.1 mg a.i./L)

And mortality of 20 and 85% was observed to the 2.1 and 3.8 mg/L treatment levels, respectively. 100% mortality was observed among fish exposed to the 6.6 mg/l at 24 hours of exposure.

Based on mean measured concentrations, the 96 hour-LC_{50} of sodium 2-biphenylate to *Oncorhyncus mykiss* under flow-through conditions was 2.6 mg/L.

Hoberg (2006b)
The acute toxicity of sodium 2-biphenylate to bluegill sunfish (*Lepomis macrochirus)* was investigated in a flow-through test at nominal substance concentrations of 1.3, 2.7, 3.6, 6.0 and 10 mg/L. The test solutions were replaced at a rate of 90 % every 9 hours. The test substances concentration was analytically verified at test initiation and test

termination by HPLC. The mean measured concentrations were 0.79, 1.7, 2.6, 4.6 and 8.0 mg/L (61, 65, 72, 77and 80 % of nominal). The test was conducted according to GLP.

Fish were exposed in groups of ten per concentration for 96 hours and mortality was recorded at 24, 48, 72 and 96 hours. At 96h of exposure, no mortality or adverse effects were observed among fish exposed to the control, 0.79, 1.7 and 2.6 mg/L. Mortality of 35% was observed among fish exposed to the 4.6 mg/L treatment level and 100% mortality was observed among fish exposed to the 8.0 mg/l at 24 hours of exposure.

Based on mean measured concentrations, the 96 hour-LC_{50} of sodium 2-biphenylate to *Lepomis macrochirus* under flow-through conditions was 5.1 mg/L.

Hoberg (2006c)
The acute toxicity of sodium 2-biphenylate to sheepshead minnow (*Cyprinodon variegatus)* was investigated in a flow-through test at nominal substance concentrations of 3.2, 5.4, 9.0, 15 and 25 mg/L. The test solutions were replaced at a rate of 90 % every 9 hours. The test substances concentration was analytically verified at test initiation and test termination by HPLC. The mean measured concentrations were 1.6, 3.3, 6.7, 12 and 20 mg/L (52, 62, 75, 78 and 80 % of nominal). The test was conducted according to GLP.

Fish were exposed in groups of ten per concentration for 96 hours and mortality was recorded at 24, 48, 72 and 96 hours. At test termination, no mortality or adverse effects were observed among fish exposed to the control, 1.6 and 3.3 mg/L. 100% mortality was observed in 12 and 20 mg/L treatments at the 24-hour observation interval.

Observations: the protocol states that total dissolved oxygen concentration will not be allowed to drop below 75% of saturation during the test. At 24 hours of exposure, dissolved oxygen concentrations in replicates A and B of the 12 and 20 mg a.i./L treatment levels were 70, 70, 60 and 60% of saturation, respectively, which is slightly below the required level of 75% saturation. Many of the fish at these treatment levels exhibited adverse effects (i.e., loss of equilibrium) at test initiation. In addition, 100% mortality was observed in both treatment levels at the 24-hour observation interval. Therefore, the low dissolved oxygen readings are believed to be the result of bacterial growth from the dead fish in the solutions and had no effect on the observed mortality.

Based on mean measured concentrations, the 96 hour-LC_{50} of sodium 2-biphenylate to *Cyprinodon variegatus* under flow-through conditions was 5.1 mg/L.

2.9.2.2.2 Acute (short-term) toxicity to aquatic invertebrates

Three studies were available to assess the acute toxicity of OPP on aquatic invertebrates:

Dill D., *et al.*, (1985).
Juvenile *Daphnia magna* were exposed to six concentrations of 0.78, 1.3, 2.2, 3.6, 6.0 and 10.0 mg a.i./L of *ortho*-Phenylphenol, in a static test system for 48 hours according to an ASTM Guideline (Standard E729-80). The test was conducted under GLP.

Mortality of daphnids was recorded at 24 and 48 hours and showed a clear dose-response relationship, i.e., no daphnids died in the lowest concentration, while no *Daphnia* survived in the highest concentration. Concentrations were measured at day 0 and at day 2 and ranged between 94 and 100% of the nominal concentration. This indicates that the test substance was stable for the duration of the study. The results are based on nominal concentrationsand the 48-hour EC50 of ortho-phenylphenol to Daphnia magna under static conditions was 2.7 mga.i./L. The study is considered acceptable.

Kühn, R. (1988)

A broad study was submitted which contained the results of an acute toxicity test of *ortho*-Phenylphenol with *Daphnia magna.* The study was performed according to DIN 38412-Part 11, comparable to the OECD TG 202. The performance of the study was not stated, only the endpoints were presented.

The EC_{50} after exposure of 48 hours was estimated to be 1.5 mg a.i./L. Test concentrations were not confirmed by analytical measurements and all endpoints were based on nominal concentrations of o*rtho*-Phenylphenol.

Due to some deviations from the guideline and deficiencies found in the report documentation (e.g., non-GLP, purity of test substance not specified, insufficient description of test conditions, tested substance concentrations not reported), the study is considered as supporting information.

Ramos et al. (1998)
This study was available in the literature and included in the REACH Registration dossier of ortho-Phenylphenol. The purpose of this study was to determine the acute toxicity of polar narcotics (11 substances among which ortho-Phenylphenol was included) to three aquatic species (*Poecilla reticulata*, *Daphnia magna* and *Lymnaea stagnalis*) and to determine their lethal body burdens. Finally, the results are compared to the hydrophobicity of the chemicals. Only the acute toxicity outcomes of ortho-Phenylphenol towards invertebrates are considered in the dossier. The acute toxicity of ortho-Phenylphenol (99.5%) to *Daphnia magna* and other species was tested in a static trial according to OECD 202. The test was not conducted under GLP.

The test system comprised five treatment concentrations and a negative control. Two replicates were included per treatment level. Test nominal concentrations were selected on the basis of EC50 values collected from literature or QSAR estimations from 4xEC50 to EC50/4, but the exact values were not reported. The daphnids were cultured at 18-20 ºC under a 12-h photoperiod. 10 daphnids (24-h old) were used per replicate in the mortality tests.

During the study the pH ranged between 8.0-8.3 and dissolved oxygen between 8.1-9.7 mg/L. Water samples were not analysed for concentration verification of ortho-Phenylphenol. Therefore, nominal concentrations were used to estimate the endpoints. The 48-h EC50 was estimated to be 2.7 mg/L.

Deviations: absence of information on nominal concentrations, lack of verification of tested concentrations and absence of information of controls mortality; the test was not conducted following GLP. This study is considered as supporting information.

To assess the acute toxicity of SOPP on aquatic invertebrates two studies were available:

The acute toxicity of sodium salt orthophenyl phenol to mysids *Americamysis bahia* and *Crassostrea virginica* was determined in a 96-hour flow-through test according to FIFRA Guideline Number 72-3; OPPTS Draft Guideline 850.1035 (Hoberg J.R., 2006d and Cafarella M.A., 2006). The validity criteria according to OCSPP 850.1035 (2016) were met in both studies. The 96-hour LC_{50} of sodium salt orthophenyl phenol in the mysid (*Americamysis bahia*) was 0.32 mg a.s./L and in Eastern Oyster (*Crassostrea virginica*) was 3.4 mg a.s./L both based on mean measured concentrations.

Hoberg (2006d).
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 test followed guideline EPA OPPTS 850.1035 and to FIFRA Guideline Number 72-3, and it was conducted with GLP compliance. 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).

Mysids were exposed in groups of ten per concentration for 96 hours. Mortality, abnormal behavior 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 naturally occurring variability for acute tests and not toxicant-related. No mortality or sublethal effects were observed among mysids exposed to the control.

Based on the mean measured concentrations, LC50 (96 h) of 0.32 mg a.i./L was determined. The study is considered acceptable.

2.9.2.2.3 Acute (short-term) toxicity to algae or aquatic plants

The effects of OPP on algal growth have been determined in threestudies:

Hick, S. (2002)
A study was undertaken to determine the effects of OPP on the growth of the green alga *Selenastrum capricornutum (Pseudokirchneriella subcapitata)* The study was carried out according to OECD 201, US EPA Guideline OPPTS 850.5400. The validity criteria according to OECD 201 were met.

The definitive test was initiated with 10^4 cells/mL of *Pseudokirchneriella subcapitata* exposed in triplicates in a static test system for 96 hours to nominal concentrations of 0, 0.5, 1.0, 2.0, 4.0 and 8.0 mg a.i./L. Cell numbers were determined after 24, 48, 72 and 96 hours as a base to calculate average growth rates and resulting growth inhibition of the algal culture.

Measured concentrations were in the range from 72 to 103 % of the nominal concentration during the test. The lowest values were found at 96 hours. All endpoints are based on mean measured concentrations.

The calculated average growth rates decreased in a dose dependent manner. The 72 h and 96 h NOEC values were 0.468 and 0.432 mg/L, respectively, based on the lack of a statistical growth inhibition at these concentrations. Based on measured concentrations of orthophenylphenol, the biomass growth 72- and 96- hour EbC50 values for *Pseudokirchneriella subcapitata* were 1.35 and 1.32 mg ai/L, respectively (calculated as the mean area under the growth curve). Based on growth rate, the 72- and 96-hour ErC50 values were 3.57 and 3.78 mg a.i./L, respectively.

The study is considered acceptable. Although measured concentrations of *ortho*-Phenylphenol at 96 hours were velow 80% of nominal concentrations (i.e., 72%), relevant values of this study are those estimated at 72 hours.

Caspers (1989c).
A second algae species was tested (*Scenedesmus subspicatus*) despite OPP did not show herbicidal activity. The study was conducted according to the German testing procedure DIN 38412 L9 (1989), comparable to OECD 201 (1984). and was not conducted under GLP. The algae were exposed in triplicates to seven concentrations of *ortho*-Phenylphenol (0.1, 0.32, 1.0, 3.2, 10.0, 32.0 and 100.0 mg/L) in a static 72-hour toxicity test.

The endpoints were estimated on the basis of nominal concentrations, since analytical verification of concentrations was not conducted. Based on the mean area under the growth curve, the 72-hour E_bC_{50} value was estimated to be 0.85 mg/L and the 72-hour E_bC_{10} was 0.38 mg/L. Based on growth rate, the 72-hour E_rC_{50} and E_rC_{10} values were estimated to be 0.98 and 0.4 mg/L, respectively.

There were some deviations from the OECD 201: test medium was not specified; tested concentrations were not confirmed by analytical measurements; the purity of *ortho*-Phenylphenol was not declared; the pH of the control increased more than 1.5 units during the test. The validity criteria according to OECD 201 could not be checked Therefore, the study is considered as supporting information.

Ramos et al. (1999)
This study was available in the literature and included in the REACH Registration dossier of ortho-Phenylphenol. The purpose of this study was to determine the algal growth inhibition of polar narcotics (11 substances including ortho-Phenylphenol) with the aquatic algae *Chlorella pyrenoidosa* and to estimate their lethal body burdens. Then the results were compared to the hydrophobicity of the chemicals. Only the toxicity test outcomes of ortho-

Phenylphenol towards algae are considered in the Registration dossier. The algal growth inhibition of ortho-Phenylphenol (98.5% purity) to *Chlorella pyrenoidosa* was tested in a trial according to OECD TG 201.

The test system comprised five treatment concentrations and a negative control. Treatment concentrations were not reported. Three replicates were included per treatment level. The inoculum added to the system had ca. 2·106 cell/mL.

During the study the pH was ca. 7.4 and temperature was 22ºC. Measured concentrations of the 11 chemicals used varied from 44 to 100% of nominal. No specific information was reported for ortho-Phenylphenol. The average population growth rate of the controls was 1.0 day-1. This is in line with one of the validity criteria of the protocol which requires a specific growth rate of at least 0.92 day-1.
Measured concentrations were used to estimate the endpoints. The 72-h ErC50 and ErC10 were estimated to be 5.0 and 3.8 mg/L, respectively. The 72-h NOEC and LOEC were 0.35 and 1.0 mg/L, respectively.

Deviations: absence of information on tested concentrations (both nominal and measured) and other details of the test system; the test was not conducted following GLP. The outcomes of this test are part of a broader study which was not conducted for regulatory purposes. This study is considered as supporting information.

Three studies on effects of sodium salt orthophenyl phenol (SOPP) on algal growth were available:

The effects of sodium salt orthophenyl phenol on the growth of the blue-green alga *Anabaena flos-aquae,* freshwater diatom *Navicula pelliculosa* and on the marine diatom *Skeletonema costatum* were determined in a 96-hour static test according to OPPTS Draft Guideline 850.5400 (Hoberg J.R., 2006e, 2006f and 2006g). The test substance showed an algistatic, rather than algicidal effect on the growth of the three algae species.

In the three studies, several validity criteria according to OECD 201 were not fulfilled and the results were considered as supporting information.

Hoberg (2006e).
A study was undertaken to determine the effects of sodium 2-biphenylate on the growth of the blue-green alga *Anabaena flos-aquae* in a static test desing. The study was carried out according to OECD 201, US EPA Guideline OPPTS 850.5400 and it was conducted with GLP compliance.

The algae were exposed in triplicates to nominal concentrations of 0.0098, 0.039, 0.16, 0.63, 2.5 and 10 mg a.i./L in a static test system for 72 hours. The test substance concentration was analytically verified at test initiation and test termination by HPLC. The mean measured concentrations were 0.0052, 0.034, 0.15, 0.59, 2.4 and 9.6 mg a.i./L (53, 87, 94, 93, 96, and 96% of nominal). Algae were exposed in a continuous illumination for 72 hours, pH was maintenced from 6.8 to 7.8 and temperature from 22 to 23°C. Effect parameters were measured by a hemacytometer every 24 hours.

Based on the growth rate an ErC50 (72 h) of 5.9 mg a.i./L (arithmetic mean measured) was determined. The reported NOErC (72 h) is 2.4 mg a.i./L (arith. mean measured)

Hoberg (2006f).
A study was undertaken to determine the effects of sodium 2-biphenylate on the growth of the freshwater diatom *Navicula pelliculosa* in a static test desing. The study was carried out according to OECD 201, US EPA Guideline OPPTS 850.5400 and it was conducted with GLP compliance.

The algae were exposed in triplicates to nominal concentrations of 0.0098, 0.039, 0.16, 0.63, 2.5 and 10 mg a.i./L in a static test system for 72 hours. The test substance concentration was analytically verified at test initiation and test termination by HPLC. The mean measured concentrations were 0.0089, 0.035, 0.15, 0.59, 2.4 and 9.6 mg a.i./L (91, 91, 93, 94, 96, and 96% of nominal). Algae were exposed in a continuous illumination for 72 hours, pH was maintenced from 7.2 to 9.1 and temperature of 24°C. Effect parameters were measured by a hemacytometer every 24 hours.

Based on the growth rate an ErC50 (72 h) of 5.7 mg a.i./L (arithmetic mean measured) was determined. The reported NOErC (72 h) is 0.59 mg a.i./L (arithmetic mean measured)

Hoberg (2006g).
A study was undertaken to determine the effects of sodium 2-biphenylate on the growth of the marine diatom *Skeletonema costatum* in a static test desing. The study was carried out according to OECD 201, US EPA Guideline OPPTS 850.5400 and it was conducted with GLP compliance.

The algae were exposed in triplicates to nominal concentrations of 0.0024, 0.0098, 0.039, 0.16, 0.63, 2.5 and 10 mg a.i./L in a static test system for 72 hours. The test substance concentration was analytically verified at test initiation and test termination by HPLC. The mean measured concentrations were 0.0019, 0.0071, 0.034, 0.15, 0.60, 2.4 and 9.8 mg a.i./L (80, 72, 88, 91, 94, 95, and 98% of nominal). Algae were exposed in a pohotoperiod of 14-hour light and 10-hour darkness for 72 hours, pH was maintenced from 7.9 to 8.8 and temperature 20 - 21°C. Effect parameters were measured by a hemacytometer every 24 hours.

Based on the growth rate an ErC50 (72 h) of 7.4 mg a.i./L (arithmetic mean measured) was determined. The reported NOErC (72 h) is 2.4 mg a.i./L (arithmetic mean measured)

Hoberg 2006h

Additionally, a study on effects of sodium salt orthophenyl phenol on aquatic macrophytes was available despite OPP/SOPP is not an herbicide or a plant growth regulator and OPP does not have herbicidal activity.
Hoberg 2006h investigated the effects of sodium salt orthophenyl phenol on the growth of the duckweed *Lemna gibba*. The study was conducted according to OPPTS Draft Guideline 850.4400; OECD Proposed Guideline 221. The validity criterion according to OECD 221 (2006) were fulfilled. The 7-day EC_{50} values based on frond density, growth rate and frond biomass (dry weight) were determined as 6.2 mg a.s./L, > 9.4 mg a.s./L and 7.7 mg a.s./L, respectively. The 7-day NOEC based on frond density, growth rate and frond biomass (dry weight) were all found to be 2.3 mg a.s./L.

2.9.2.2.4 Acute (short-term) toxicity to other aquatic organisms

Not relevant

2.9.2.3 Long-term aquatic hazard [equivalent to section 11.6 of the CLH report template]

Table 2.9.2.3:1 Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference
Long term and chronic toxicity to fish. Guideline similar to OECD 234, 229 and 230	*Pimephales promelas*	OPP Purity: 99.9%	21d-NOEC = 0.036 mg/L (mm)	Accepted	General validity criteria were met	Caunter J., Williams T., (2002),
Long term and chronic toxicity to aquatic invertebrates OECD 211	*Daphnia magna*	OPP Purity: 99.85%	**21d-NOEC = 0.006 mg/L (mm)**	Accepted	validity criteria were met	Bruns, (2001)

Long term and chronic toxicity to aquatic invertebrates Draft 4XI/681/86, (EG Brief: Brussels 24/09/1987) Guideline similar to OECD 211	*Daphnia magna*	OPP Purity: not reported	21d-NOEC = 0.0075 mg/L (nom)	Not relevant	OECD 211 validity criteria were not met.	Caspers, (1989b)
Toxicity to algae or other aquatic plants. OECD 201, US-EPA OPPTS 850.5400	*Selenastrum capricornutum*	OPP, Purity: 99.91%	72h-NOEC = 0.468 mg/L (mm)	Accepted	validity criteria were met.	Hicks, S. (2002)
Toxicity to algae or other aquatic plants. German testing procedure DIN 38412 L9 (1989)	*Scenedesmus subspicatus*	OPP, Purity: not reported	72h-ErC10 = 0.4 mg/L (nom)	Supporting information	OECD 201 validity criteria could not be checked	Caspers (1989c)
Toxicity to algae or other aquatic plants. OECD 201	*Chlorella pyrenoidosa*	OPP. Purity: 98.5%	72h-ErC10= 3.8 mg /L	Supporting information	Not GLP.	Ramos et al. (1999)
Toxicity to algae or other aquatic plants. OECD 201, US-EPA OPPTS 850.5400	*Anabaena flos-aquae*	SOPP Purity: 71.48%	72h-NOEC = 2.4 mg/L (mm)	Supporting information	validity criteria were not met.	Hoberg (2006e)
Toxicity to algae or other aquatic plants.	*Naviculla pelliculosa*	SOPP Purity: 71.48%	72h-NOEC = 0.59 mg/L (mm)	Supporting information	validity criteria were not met.	Hoberg (2006f)

OECD 201, US-EPA OPPTS 850.5400						
Toxicity to algae or other aquatic plants. OECD 201, US-EPA OPPTS 850.5400	*Skeletonema castatum*	SOPP Purity: 71.48%	72h-NOEC = 2.4 mg/L (mm)	Supporting information	validity criteria were not met.	Hoberg (2006g)
OECD 219	*Chironomus riparius*	OPP Purity: 100%	NOEC = 1.85 mg /L (mm)	Accepted	validity criteria were met	Egeler P., Gilberg D., (2005)

2.9.2.3.1 Chronic toxicity to fish

Caunter J., Williams T., **(2002).**
A study was carried out to determine the effects of OPP on the reproduction of the fathead minnow *Pimephales promelas* under flow-through conditions according to Harries *et al.*, 2000, Development of a reproductive performance test for endocrine disrupting chemicals using pair-breeding fathead minnows (*Pimephales promelas*) (Environmental Science Technology 34, 3003-3011). This guideline is not directly comparable to any current OECD guidelines. The test carried out was similar in some respects to OECD Guidelines 234: Fish Sexual Development Test, 229: Fish Short Term Reproduction Assay, and 230: 21-day Fish Assay. In terms of validity criteria of the OECD guidelines above, this test was considered valid. The overall NOEC was 36 µg OPP/L (mean measured) based on effects observed in fecundity and hatchability.

Reproductively active adult fish were exposed to four concentrations of *ortho*-Phenylphenol (1.0, 5.0, 50 and 500 µg/L) for 21 days. One breeding pair of fish (male and female) was tested in each tank (6 replicates per treatment). There was a negative and a positive control (17α-ethynylestradiol).

The biological parameters observed daily during the exposure phase were the number of spawnings, number of eggs spawned and number of eggs per spawning (egg batch size). Viability of resultant embryos was assessed in separate tanks held in the same treatment regime to which the adults were exposed. The percent hatchability of fertilised eggs was determined. When hatching was complete, the F1 generation larvae were discarded. After the exposure phase, length and weight of adult fish were measured; plasma vitellogenin was analysed; the gonadosomatic index was determined; and histopathology analysis was carried out.

Mean measured concentrations ranged from 59-81% of nominal. Therefore, the endpoints were based on mean measured concentrations.
Comparison of egg production, batch size and egg batch before and after exposure to the test substance showed a trend indicating a reduction in the spawning, number of eggs, batch size and egg batch in the 5 and 50 µg a.i./L treatments. Besides that, these changes are not statistically significant carefully interpretation is required. The overall 21 d-NOEC for reproductive parameters was determined to be 0.036 mg/L.

Measurements of GSI and induction of VTG indicated no effects up to and including the highest concentration tested (0.293 mg/L). With regard to the induction of the biomarker vitellogenin as an early indicator of possible endocrine modulation, no substance-related effects were noted compared to the positive control 17α-ethynylestradiol.

2.9.2.3.2 Chronic toxicity to aquatic invertebrates

Long-term toxicity of OPP to aquatic invertebrates has been determined in two studies:

Bruns (2001).
The influence of ortho-Phenylphenol 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. The test media was verified by HPLC. During the test a temperature range of 18 - 22°C was to be maintained in the test vessels, with a maximum temperature fluctuation of +/- 2°C in each individual test. Test vessels must not be aerated during the test. A photoperiod of 8 hours darkness and 16 hours light is maintained.

Concentrations were analysed during the study. Arithmetic mean measured concentrations were included in the laboratory report (i.e., 0.009, 0.022 and 0.07 mg/L). The results are accepted but measured concentrations are recalculated on the basis of geometric means. 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.

Due to the differences among the measured concentrations, having found some values below the LOQ, the geomean is a more accurate mean than the arithmetric mean. Actually, in BPR guidance Vol IV, part B+C, section 3.10.2, for the assessment of the ecotoxicological endpoints for active substances that degrade rapidily in a test system, if the measured concentrations are available, the geometric mean of the concentratios may be calculated as an approximation of the actual exposure.

Resulting values were: LOECreproduction = 0.011 mg/L, LOECmortality ≥ 0.024 mg/L, NOECreproduction = 0.006 mg/L, NOECmortality ≥ 0.024 mg/L.

Deviations: Three test substance concentrations were tested instead of five; according to the guideline the deviation from the nominal or measures initial concentration must be ± 20% and in the test, masured concentrations ranged from 70 to 90 % of the nominal values. These deviations were not considered to have affected the outcome of the study. Validity criteria of the test (mortality rate in controls < 20 %, living offspring per daphnia in controls > 60) were fulfilled.

The study is considered acceptable.

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Caspers (1989b).
A study on the long-term toxicity of ortho-Phenylphenol to Daphnia magna, after 21 days exposure under semi-static conditions, was performed according to the Draft Guideline 4XI/681/86 (Prolonged Toxicity Study with Daphnia magna: Effects on Reproduction. EG Brief: Brusssels 24/09/1987). The study was not conducted under GLP.

Young parthenogenetic female Daphnia magna, aged between 6 and 24 hours, were exposed to three nominal concentrations of ortho-Phenylphenol (0.0075, 0.075 and 0.75 mg/L). After a 21-d exposure period, the total number of offspring per parent animal was assessed in order to determine effect concentrations. Additionally, parental mortality was recorded.

The recorded pH values and oxygen concentrations were satisfactory maintained throughout the study period. Concentrations of ortho-Phenylphenol in the water were measured initially (before starting of the study) (they ranged from 83 to 97% of nominal) and after 48 hours (the test substance was either not detected or present in only trace quantities). However, it is not clear whether the test substance was measured during this test. The endpoints were estimated on the basis of nominal concentrations.

One daphnid is tested per vessel. No animal died during the study at concentrations equal to or lower than 0.075 mg/L, whereas all daphnids exposed to 0.75 mg/L were dead. The resulting endpoints were the 21-d EC50,reproduction of 0.075-0.75 mg/L and EC50,mortality of 0.075-0.75 mg/L.

There were some significant deficiencies, i.e.; the purity and lot number of the test substance was not specified; the regime of medium renewal was not reported; there were no analytical determination of test substance

concentrations; three concentrations were tested instead of 5 as indicated in EOCD 211; the number of replicates was not reported; the validity criterion of OECD TG 211 in relation to the mean number of live offspring produced per parent animal surviving at the end (i.e., ≥ 60) was not fulfilled (i.e., 44.4 juveniles/parent was reported).
Therefore, the results of this study are not considered reliable

2.9.2.3.3 Chronic toxicity to algae or aquatic plants

Please refer to point 2.9.2.2.3 where the summaries of toxicity test on algae are included.

2.9.2.3.4 Chronic toxicity to other aquatic organisms

A study of the toxicity of OPP to the sediment dweller Chironomus riparius was provided despite OPP is not an insect growth regulator.

Egeler, P., Gilberg, D. (2005)
The long-term toxic effects of *ortho*-Phenylphenol to the larvae of *Chironomus riparius* were investigated in a static study according to OECD TG 219 and following GLP. The larvae were exposed to the tested substance for 28 days. Emergence ratio and development rate were the observational parameters.

In two preliminary range finding tests with spiked sediment and spiked water, it was found that the test organisms exposed to spiked water were affected at considerably lower concentrations than the larvae exposed to spiked sediment. Therefore, the definitive test was performed with spiked water (OECD 2019).

ortho-Phenylphenol (100% purity) was added to the vessels by spiking the water. Nominal concentrations were 0.25, 0.5, 1, 2 and 4 mg/L. The substance moved from the overlying water to the sediment during the test. The recoveries of *ortho*-Phenylphenol decreased throughout the test period. The average recovery of the initially measured concentrations (1 hour after addition to the vessel) was 92.5% of the nominal concentrations and the endpoints were obtained based on these concentrations. After 7 days concentrations declined to 34-55% of nominal in the water phase. By the end of the test, only 2.6 - 3.2 % were measured.

With respect to the emergence ratio, the test showed a clear dose-response relationship, thus EC_x values were estimated. For the development rate there was also a dose-response relationship however EC_x values could not be calculated since the inhibition of the development rate was not higher than 17% and 23% of the controls for females and males, respectively. NOEC and LOEC values were determined for both parameters. The endpoints for *Chironomus riparius* exposed to *ortho*-Phenylphenol after 28 days were: EC_{50}= 3.35 mg/L for emergence ratio, NOEC= 1.85 mg/L for emergence ratio and development rate, and LOEC= 3.70 mg/L for emergence ratio and development rate (results based on the measured test item concentrations).

The test showed minor deviations from the testing protocol. However, the validity criterion was fulfilled, since more than 80% of the control larvae had emerged before day 23.

The study is considered acceptable.

2.9.2.4 Comparison with the CLP criteria

2.9.2.4.1 Acute aquatic hazard

Table 2.9.2.4.1-1: Summary of information on acute aquatic toxicity relevant for classification

Method	Species	Test material	Results	Remarks	Reference
Acute toxicity to fish ASTM Standard E729-80	*Oncorhynchus mykiss*	OPP Purity: 99.25%	96h-LC_{50} = 4.0 mg/L (nom)	Accepted	Dill D., *et al.*, (1985)

Guideline similar to OECD 203					
Acute toxicity to fish Guideline similar to OECD 203	Rainbow trout (*Oncorhynchus mykiss*)	SOPP Purity: 71.48%	96h-LC_{50} = 2.6 mg/L	Accepted	Hoberg (2006a)
Acute toxicity to aquatic invertebrates Guideline similar to OECD 202	*Daphnia magna*	OPP	48h-EC_{50} = 2.7 mg/L (nom)	Accepted	Dill D., *et al* (1985)
Acute toxicity to aquatic invertebrates OCSPP 850.1035 (2016)	Mysid (*Americamysis bahia*)	SOPP Purity: 74.18%	**96h-LC_{50} = 0.32 mg/L (mm)**	Accepted	Hoberg (2006 d)
Acute toxicity to algae or other aquatic plants OECD 201, US-EPA OPPTS 850.5400	*Scenedesmus subspicatus*	O-phenylphenol (OPP). Purity: 99.91%	72h-ErC50 = 3.57 mg/L (nom)	Accepted	Hicks (2001)
~~2.9.2.4.1.1.1.1~~	~~2.9.2.4.1.1.1.2~~	~~2.9.2.4.1.1.1.3~~	~~2.9.2.4.1.1.1.4~~		~~2.9.2.4.1.1.1.5~~

Acute aquatic hazard
Full acute data set was available for *ortho*-Phenylphenol and its sodium salt as there were acute studies on fish, aquatic invertebrates and algae and aquatic plants, covering the three trophic levels (see Table 2.9.2.2). Taking into account the lowest and most reliable values for these three tropich levels, invertebrates are the most sensitive trophic level with the 96h-EC_{50} of 0.32 mg/L determined with *Americamysis bahia* (see Table 2.9.2.4.1-1 above).

For classification of a substance in relation to acute aquatic hazard, table 4.1.0 (a) of Annex I of Regulation (EC) No. 1272/2008 should be used. The acute endpoint selected has to be compared with the cut-off value (acute toxicity values ≤ 1 mg/l).-The 96-h EC_{50} of 0.32 mg/L is ≤ 1 mg/L. Therefore *ortho*-Phenylphenol should be classified as Aquatic Acute 1. The corresponding Multiplication factor (M-factor) should be 1, since $0.1 < E_rC_{50} \leq 1$.

The current entry in Annex VI of *ortho*-Phenylphenol already includes category Aquatic Acute 1. It is proposed to keep the same hazard category and to add M-factor of 1.

2.9.2.4.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Table 2.9.2.4.2-1: Summary of information on long-term aquatic toxicity relevant for classification

Method	Species	Test material	Results	Remarks	Reference
Long term and chronic toxicity to fish	*Pimephales promelas*	OPP	NOEC = 0.036 mg/L (mm)	Accepted	Caunter J., Williams T., 2002

Guideline similar to OECD 234, 229 and 230					
Long term and chronic toxicity to aquatic ivertebrates OECD 211	*Daphnia magna*	OPP Purity: 99.85%	**NOEC = 0.006 mg/L (mm)**	Accepted	Bruns (2001)
Long term and chronic toxicity to algae or other aquatic plants OECD 201, US-EPA OPPTS 850.5400	*Selenastrum capricornutum*	OPP, Purity: 99.91%	72h-NOEC = 0.468 mg/L (mm)	Accepted	Hicks, (2002)
Toxicity to sediment-dwelling OECD 219	*Chironomus riparius*	OPP Purity: 100%	NOEC = 1.85 mg /L (mm)	Accepted	Egeler, P., Gilberg, D. (2005)

Degradability

ortho-Phenylphenol can be considered to be readily biodegradable since there were several ready biodegradation studies available which demonstrated a high level of degradation within the 10-d window. Therefore, *ortho*-Phenylphenol can also be considered as rapidly degradable substance.

Bioaccumulation

The log K_{OW} of *ortho*-Phenylphenol is 3.18, thus it is below the threshold of ≥ 4 of potentially bioaccumulative substances. In addition, the experimental BCF in fish normalised by the lipid content was determined to be 115. This is below the threshold of ≥ 500 of bioaccumulative substances. Therefore *ortho*-Phenylphenol is not a bioaccumulative substance.

Chronic aquatic hazard

A full set of chronic data for three trophic levels is available. The chronic toxicity in fish is covered in a long-term test with *Pimephales promelas*.The chronic toxicity in aquatic invertebrates is covered in a long-term test with *Daphnia magna.* Additionally, a study of sediment-dwelling (*Chironomus riparius*) organism was also assessed. Long-term toxicity data for 2 algal species are available. Thus, adequate chronic data are available for three trophic levels, fish, algae and invertebrates.

The lowest chronic endpoint is the 21-d NOEC of 0.006 mg/L on *D. magna*. Since the substance is rapidly degradable and there is adequate chronic data for crustaceans, the chronic NOEC should be compared to the threshold values based on chronic data (table 4.1.0 (b)(ii)). The 21-d NOEC of 0.006 mg/L is <0.01 mg/L. Thus *ortho*-Phenylphenol should be classified as Chronic 1.

The corresponding M-factor proposed should be 1, since 0.001 mg/L < NOEC = 0.006 mg/L ≤ 0.01 mg/L and the substance is rapidly degradable.

2.9.2.5 Conclusion on classification and labelling for environmental hazards

Taking into account all the information and the assessment summarized in the previous sections 2.9.2.4.1 and 2.9.2.4.2, the following classification class and category can be concluded for this active substance 2-Phenylphenol and its salt, in accordance with Regulation (EC) 1272/2008:

2-phenylphenol and sodium salt 2-phenylphenol

CLP Annex ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification[1]	Reason for no clasification[2]
4.1	Hazardous to the aquatic environment	Aquatic Acute 1 H400 Aquatic Chronic 1 H410	M-factor = 1 M-factor = 1	Aquatic Acute 1	-
5.1	Hazardous to the ozone layer	-	-	-	Data lacking

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Warning

Hazard statements: Very toxic to aquatic life with long lasting effects (H410)

Precautionary statements:

P273: Avoid release to the environment
P391: Collect spillage
P501: Dispose of contents/container in accordance with national hazardous waste regulations

Pictogram: GSH09

The following additional statements are recommended.

- EUH401: To avoid risks to human health and the environment, comply with the instructions for use.

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)
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)
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 ($0.1 < EC_{50} \leq 1$). 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.**

2.9.3 Summary of effects on arthropods

OPP is applied as a post-harvest application. The application takes place within packing houses. No application is made outdoors. There is no application to crops and no spray drift to surrounding non-target plants. There will be no exposure to non-target arthropods during application to harvest fruits. There will be no exposure to flowers, therefore there will be no residues of OPP in pollen or nectar. This is in compliance with Regulation (EC) 283/2013, which states that studies are not required where plant protection products containing the active substance are for exclusive use in situations where bees are not likely to be exposed. Nevertheless, a study was undertaken to determine the contact toxicity of OPP to the honey bee *Apis mellifera* according to OECD 204 guideline. The validity criteria were met and the study was considered as valid. The honey bee *Apis mellifera* the 48 hour LD_{50} was >100 µg OPP/bee and the NOEC was 25 µg OPP/bee.

Table 2.9.3-1: Summary of arthropods toxicity endpoints

Test type	Test species	Endpoint
Acute contact toxicity	Apis mellifera	LD_{50}> 100 µg OPP/bee NOEC = 25 µg OPP/bee

2.9.4 Summary of effects on non-target soil meso- and macrofauna

Exposure to the environment is not expected from the use of OPP in accordance with the representative use. Nevertheless, a study was carried out to determine the acute toxicity of OPP to the earthworm Eisenia fetida

according to OECD 207 (Moser T., 2004). 40 earthworms were tested per test substance concentration. The test substance concentrations were 0 (water control and acetone control), 62.5, 125, 250, 500, 1000 mg OPP/kg soil dw. A toxic reference, chloroacetamide, was also tested at 5, 10, 20 and 40 mg chloroacetamide/kg soil dw. Earthworms were exposed to test substance for 14 days. The NOEC was 125.0 mg OPP/kg soil dw. The LC_{50} was calculated as 198.2 mg OPP/kg soil dw.

Table 2.9.4-1: Summary on non-target soil meso- and macrofauna

Test type	Test species	Endpoint
Acute toxicity, 14d	*Eisenia fetida*	EC_{50}corr = 99.1 mg a.s./kg soil dw NOECcorr = 62.5 mg a.s./kg soil dw

2.9.5 Summary of effects on soil nitrogen transformation

Two studies of effects on soil nitrogen transformation were available:
A study was conducted to determine the effects of OPP on nitrogen transformation in soil according to OECD 216 (Schulz L., 2012). The study was considered valid. The test item caused a maximum inhibition of -60.4% and -56.8% at 1000 mg/kg dw soil 28 days and 100 days after application, respectively. The NOEC was determined as 300 mg/kg dw soil on days 28 and 100 and the EC_{50} was 633.5 mg/kg dw soil on day 28 and 829.1 mg/kg dw soil on day 100.
A second study was carried out according to OECD 216 and 217 to determine the effects of OPP on nitrogen transformation in soil (Reis K., 2007). The effects on carbon transformation were also determined, but were not reported in this submission. The validity criteria were met. The test item 2-Phenylphenol had no detrimental effect on soil microbial respiration and nitrogen transformation after 28 days of incubation, up to a concentration of 1.0 mg/kg dry soil. The NOEC was ≥1.0 mg OPP/kg soil dw.

Table 2.9.5-1: Summary of effects on soil nitrogen transformation

Test design	Test species	Endpoint
28 d nitrogen transformation	Soil nitrogen microorganism	NOER = 300 mg a.s./kg soil dw
28 d nitrogen transformation	Soil nitrogen microorganism	NOER ≥ 1 mg a.s./kg soil dw

2.9.6 Summary of effects on terrestrial non-target higher plants

A study was conducted to determine the effects of OPP on seedling emergence and growth of non-target plants according to OECD 208 (Bützler R., Meinerling M., 2008). The tested spescies were *Glycine max Brassica napus* and *Avena sativa.* The most sensitive plant was *Avena sativa*, with a NOEC of 12.5 mg OPP/kg soil dw and an EC_{50} of 53.9 mg OPP/kg soil dw. The NOEC of *Brassica napus* was determined as 25.0 mg OPP/kg soil dw and the EC_{50} was 62.9 mg OPP/kg soil dw. The least sensitive plant was *Glycine max,* with a NOEC of 25.0 mg OPP/kg soil dw and an EC_{50} of 89.7 mg OPP/kg soil dw. No statistically significant mortalities or reductions in germination rate were observed in any species. The study was well conducted. However, only three species were tested. According to Regulation 283/2013, the dose-response test should be carried out on a selection of 6 to 10 monocotyledon and dycotiledon plant species representing as many taxonomic group as possible. Therefore, the most sensitive specie can not be stablished and this information is considered supportive only.

Additionally, two studies were available to determine the effects of SOPP on Seedling Emergence and Vegetative Vigour of Rice (*Oryza sativa*) according to OPPTS Draft Guidelines 850.4100 and 850.4225 (Teixeira, D., 2006a and 2006b). Exposure of *Oryza sativa* to sodium salt orthophenyl phenol at 1000 mg a.s./L did not cause adverse effects ≥ 25% on seedling emergence and growth (shoot length and shoot dry weight), i.e. EC_{25} and EC_{50} are > 1000 mg a.s./L (ER_{25} and ER_{50} > 7131 g a.s./ha). Exposure of *Oryza sativa* to sodium salt orthophenyl phenol at 1000 mg a.s./L did not cause adverse effects ≥ 25% on vegetative vigour (shoot length and shoot dry weight), i.e. EC_{25} and EC_{50} are > 1000 mg a.s./L (ER_{25} and ER_{50} > 233.9 g a.s./ha). Since, only one *specie* was tested, this information is considered as supplemental only.

Table 2.9.6-1: Summary of effects of 2-phenylphenol on terrestrial non-target higher plants

Test design	Test species	Endpoint

Effects on seedling emergence and growth 14 days after emergence	*Avena sativa*	ER_{50} = 53.9 mg OPP/kg soil dw NOEC = 12.5 mg OPP/kg soil dw
	Brassica napus	ER_{50} = 62.9 mg OPP/kg soil dw NOEC = 25.0 mg OPP/kg soil dw
	Glycine max	ER_{50} = 89.7 mg OPP/kg soil dw NOEC = 25.0 mg OPP/kg soil dw

Table 2.9.6-2: Summary of effects of sodium salt 2-phenylphenol on terrestrial non-target higher plants

Test design	Test species	Endpoint
Effects on seedling emergence and growth 14 days after emergence	*Oryza sativa*	EC_{25}/EC_{50} >1000 mg SOPP/L ER_{25}/ER_{50} >7131 g SOPP/kg soil dw
Effects on Vegetative Vigour in a 14-day test	*Oryza sativa*	EC_{25}/EC_{50} >1000 mg SOPP/L ER_{25}/ER_{50} > 233.9 g SOPP/kg soil dw

2.9.7 Summary of effects on other terrestrial organisms (flora and fauna)

No further data is presented for effects on other terrestrial organisms.

2.9.8 Summary of effects on biological methods for sewage treatment

Three studies to determine the effects of OPP on sewage treatment plants were carried out:
In the first study, the effects of OPP on activated sludge were determined in accordance with OECD 303A (Stürznickel K., 2016). The test was conducted using synthetic waste water consisting of domestic waste water spiked with OPP. According to the results, it was demonstrated that OPP was completely biodegraded. Adsorption onto activated sludge was not occur. Both degradation of carbon compounds present in the wastewater sample and biological ammonium oxidation by nitrification were not inhibited by OPP.

A second study was carried out to in accordance to OECD Activated Sludge, Respiration Inhibition Test for assessment of the potential impact of chemicals on wastewater treatment systems. The objectives of the test were to determine inherent variability in active sludge respiration rate analysis, to determine the reproducibility of IC50 values for a range of reference substances, to develop appropriate statistics to predict toxic effects of chemicals and to determine the reliability of the laboratory test for predicting effects in waste water treatment facilities. The IC_{50} was 48.6 – 56.0 mg OPP/L depending on which calculation method was used.

In a third study, the toxicity of OPP to bacteria in activated sewage sludge was investigated in accordance to ISO regulation 8192-1986 (E). The respiratory rate of activated sludge mixed with nutrient solution was compared to respiratory rates of activated sludge, nutrient solution and test substance. A toxicity reference substance, 3,5-dichlorophenol was also tested, though the results were not reported. The EC_{50} was 62.2 mg OPP/L and the NOEC 32.0 mg OPP/L. The validity criteria according to OECD 209 could not be checked since there was no control test in this study and the results of the refernce substance 3,5-dichlorophenol were not reported. Therefore, this information was considered as additional.

Table 2.9.8-1: Summary of effects on biological methods for sewage treatment

Test type/organism	end point
Activated sludge	OPP was completely biodegraded. Adsorption onto activated sludge did not occur. Degradation of carbon compounds in the wastewater and biological ammonium oxidation by nitrification were not inhibited by OPP. The IC_{50} was 48.6 – 56.0 mg OPP/L.

2.9.9 Summary of product exposure and risk assessment

The risks to aquatic organisms and fish-eating terrestrial organisms from the use of OPP were calculated. These are presented in detail in Vol 3 CP section 9. Other risk assessments were not carried out, explanations are provided below.
The worst case scenario, or critical GAP, was used for the representative crop. The critical GAP is listed in the table below.

Table 2.9.9-1: OPP Critical GAP

Crop	Application timing	Nº. Applications	Application interval [days]	Max. product rate	Max. a.s. rate [g a.s./ha]	PHI [days]
Citrus fruits	Post-harvest	1	n/a	0.6 L/hL	60 g/hL	n/a

2.9.9.1 Risk assessments for birds and mammals

The risks to birds and mammals from the use of OPP has not been calculated. The proposed use of OPP in this active substance renewal submission is a post-harvest fungicidal treatment of citrus fruits. OPP is applied in a closed system, indoors. There will be no exposure to terrestrial vertebrates.
Drinking water risk assessments were not conducted for birds and mammals. The proposed use of OPP in this active substance renewal submission is a post-harvest fungicidal treatment of citrus fruits. OPP is applied in a closed system, indoors. There will be no exposure to terrestrial vertebrates. It has been proposed that surface water could be exposed via effluent exposure, therefore PECsw values have been calculated. Date on the bioconcentration of OPP in fish has been provided.
The risks to fish-eating birds and mammals have been calculated in accordance with EFSA Journal 2009;7(12):1438. The results are presented in the table below.

Table 2.9.9.1-1: Risk of secondary poisoning to fish-eating vertebrates

Organism	PECsw (µg/L)	BCF	PECfish (µg/kg)	Daily dose (µg/day)	LD_{50}/NOEL (mg/kg or mg/kg bw/d)	TER
Bird	0.4048	21.7	8.7842	1.3967	>5620	4022906
Mammal				1.2474	39	31275

The TER values are considerably above the trigger of 5, therefore the risks to fish-eating birds and mammals are acceptable.

2.9.9.2 Risk assessment to aquatic organism

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015). The exception from the guidance is the method by which PEC_{sw} values were calculated.
The proposed use of OPP in this active substance renewal submission is a post-harvest fungicidal treatment of citrus fruits. OPP is applied in a closed system, indoors. There will be no exposure to the environment. However, it has been suggested by RMS Spain that waste water from cleaning processes could enter surface waters via emission from sewage treatment plants (STP). PEC_{sw} values have been calculated from $PEC_{effluent}$ values, which were modelled using SimpleTreat version 3.1 and SimpleTreat version 4.0. More details of the PEC_{sw} calculations are provided in Vol 3 CP Section 8.
The results of the risk assessment for OPP are presented in the tables below.

Table 2.9.9.2-1: Risk assessment for aquatic organisms from use of OPP on post-harvest citrus fruits (PECsw calculated with SimpleTreat 3.1)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}
(µg/L)		4000	3.6	2700	6	3570
AF		100	10	100	10	10
RAC (µg/L)		40	0.36	27	0.6	357
SimpleTreat 3.1	**PEC_{sw} (µg/L)**	0.4048	0.0355	0.4048	0.0355	0.0355
PEC/RAC	**(Pass < 1)**	0.010	0.099	0.015	0.059	0.000

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 2.9.9.2-2: Risk assessment for aquatic organisms from use of OPP on post-harvest citrus fruits (PECsw calculated with SimpleTreat 4.0)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}
(µg/L)		4000	3.6	2700	6	3570
AF		100	10	100	10	10
RAC (µg/L)		40	0.36	27	0.6	357
SimpleTreat 4.0	**PEC_{sw} (µg/L)**	0.3865	0.0339	0.3865	0.0339	0.0339
PEC/RAC	**(Pass < 1)**	0.010	0.094	0.014	0.057	0.000

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

The risks to aquatic organisms from use of OPP as a post-harvest fungicide on citrus fruit are acceptable.

2.9.9.3 Risk assessment for non-target arthropods

The evaluation of the risk for bees and other non-target arthropods was not performed. OPP is applied as a post-harvest application. The application takes place within packing houses. No application is made outdoors. There is no application to crops and no spray drift to surrounding non-target plants. There will be no exposure to non-target arthropods during application to harvest fruits. There will be no exposure to flowers, therefore there will be no residues of OPP in pollen or nectar.

2.9.9.4 Risk assessment for soil organism

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was not performed. The proposed use of OPP in this active substance renewal submission is a post-harvest fungicidal treatment of citrus fruits. OPP is applied in a closed system, indoors. There will be no exposure to soil meso and macrofauna.

The evaluation of the risk for soil microorganisms was not performed. The proposed use of OPP in this active substance renewal submission is a post-harvest fungicidal treatment of citrus fruits. OPP is applied in a closed system, indoors. There will be no exposure to soil.

2.9.9.5 Risk assessment for non-target plants

The evaluation of the risk for non-target plants was not performed. Exposure to the environment is not expected from the use of OPP in accordance with the representative use in this dossier. Application of an aqueous solution of the formulated product to harvested citrus fruits occurs inside a packing house. No exposure to non-target plants from spray drift is expected. The waste from cleaning the application system is treated as chemical waste. No exposure to soil is expected, therefore no effects of OPP on non-target plants via translocation are expected.

2.10 ENDOCRINE DISRUPTING PROPERTIES

2.10.1 Toxicology and metabolism data

1. Gather all relevant information

Introduction into this chapter by RMS

The ED criteria according to Points 3.6.5 and 3.8.2 of Annex II of Regulation (EC) No 1107/2009, as amended by Commission Regulation (EU) 2018/605, and subsequently the ECHA/EFSA guidance document (2018), should be applied for all substances which have a pending decision on approval or renewal of approval.

The applicant has provided updated information on 2-phenylphenol (OPP) endocrine disrupting properties, mechanism of action studies, including in vitro and in vivo mechanistic data, short-term toxicity studies, long-term toxicity, carcinogenicity studies and reproductive toxicity studies (Table 2.10.1).

Furthermore, in silico data and in vitro data from the source of information US EPA Toxicity Forecaster (ToxCast) data have been provided and considered in this assessment (B.6.8.3-01).

The RMS has performed an assessment of OPP endocrine disrupting properties in line with the ECHA/EFSA guidance (2018) for the identification of endocrine disruptors.

Data were populated in the Excel template provided as Appendix E to the EFSA/ECHA guidance for the identification of endocrine disruptors (2018). According to this template each study was given an identification number (Study ID Matrix) that is important for its identification in the data-matrix of the Excel.

Table 2.10.1 Outline of dataset considered for mammalian toxicology assessment

Type of toxicity	Study	Study ID matrix	Reference	Acceptability
Short-term toxicity	Subacute oral in non-rodent (rabbit)	1	B.6.3.1-03	Supporting
	Subacute oral in non-rodent (dog)	2	B.6.3.1-04	Supporting
	Subchronic oral toxicity in rodents (rat)	3	B.6.3.2-01	Supporting (publication)
	Subchronic oral toxicity in rodents (rat)	4	B.6.3.2-02	Supporting (publication)
	Repeated dose 90-day oral toxicity study in non-rodents (dog)	5	B.6.3.2-03	Supporting
	Repeated dose 90-day oral toxicity study in non-rodents (dog)	6	B.6.3.2-04	Supporting (publication)
	Repeated dose dermal toxicity (rat)	7	B.6.3.3-01	Acceptable
	Repeated dose dermal toxicity (mouse)	8	B.6.3.3-02	Supporting
Long-term toxicity and carcinogenicity	Chronic toxicity (rat)	9	B.6.5.1-01	Supporting (publication)
	Combined chronic toxicity and carcinogenicity (2-year) study in rat	10	B.6.5.1-02	Acceptable
	Combined chronic toxicity and carcinogenicity (91-week) study in rat	11	B.6.5.2-01	Supporting (publication)
	Combined chronic toxicity and carcinogenicity (2-year) study in mouse	12	B.6.5.3-01	Acceptable
	Carcinogenicity study (102-week) in mouse	13	B.6.5.3-02	Supporting
Reproductive toxicity	Two-generation reproduction study in rat	14	B.6.6.1-01	Acceptable
	Two-generation reproduction study in rat	15	B.6.6.1-02	Acceptable
	Developmental toxicity study in rat	16	B.6.6.2-02	Supporting
	Developmental toxicity study in rat	17	B.6.6.2-01	Supporting (publication)

Type of toxicity	Study	Study ID matrix	Reference	Acceptability
	Developmental toxicity study in rabbit	18	B.6.6.2-03	Supporting
	Developmental toxicity study in rabbit	19	B.6.6.2-04	Acceptable
	Developmental toxicity study in mouse	20	B.6.6.2-05	Supporting (publication)
***In vivo* mechanistic**	Uterotrophic assay	28	B.6.8.3-06	Acceptable
	Hershberger assay	29; 30	B.6.8.3-07	Acceptable
	Pubertal Development and Thyroid Function in Intact Juvenile/ Peripubertal Female Rats	31	B.6.8.3-08	Supporting
	Pubertal Development and Thyroid Function in Intact Juvenile/ Peripubertal Male Rats	32	B.6.8.3-09	Supporting
***In vitro* mechanistic**				
	ATG_THRa1_TRANS_up	35	B.6.8.3-01	Acceptable
	NVS_NR_hTRa	36	B.6.8.3-01	Acceptable
	Tox21_TR_LUC_GH3_Agonist	37	B.6.8.3-01	Acceptable
	Tox21_TR_LUC_GH3_Antagonist	38	B.6.8.3-01	Acceptable
	NVS_GPCR_rTRH	63	B.6.8.3-01	Acceptable
	TOX21_TSHR_Agonist_ratio	64	B.6.8.3-01	Acceptable
	TOX21_TSHR_Antagonist_ratio	65	B.6.8.3-01	Acceptable
	TOX21_TSHR_wt_ratio	66	B.6.8.3-01	Acceptable
	ToxCast ER prediction model	21	B.6.8.3-01	Acceptable
	ER Binding Assay	23	B.6.8.3-02	Acceptable
	Other ER in vitro assay	24	B.6.8.3-10	Supporting (publication)
	ToxCast AR prediction model	22	B.6.8.3-01	Acceptable
	AR Binding Assay	25	B.6.8.3-03	Acceptable
	Aromatase Assay	26	B.6.8.3-04	Acceptable
	H295R steroidogenesis assay	27	B.6.8.3-05	Acceptable
	CEETOX_H295R_11DCORT_dn	39	B.6.8.3-01	Acceptable
	CEETOX_H295R_11DCORT_up	40	B.6.8.3-01	Acceptable
	CEETOX_H295R_OHPREG_dn	41	B.6.8.3-01	Acceptable
	CEETOX_H295R_OHPREG_up	42	B.6.8.3-01	Acceptable
	CEETOX_H295R_OHPROG_dn	43	B.6.8.3-01	Acceptable
	CEETOX_H295R_OHPROG_up	44	B.6.8.3-01	Acceptable
	CEETOX_H295R_ANDR_dn	45	B.6.8.3-01	Acceptable
	CEETOX_H295R_ANDR_up	46	B.6.8.3-01	Acceptable
	CEETOX_H295R_CORTIC_dn	47	B.6.8.3-01	Acceptable
	CEETOX_H295R_CORTIC_up	48	B.6.8.3-01	Acceptable
	CEETOX_H295R_CORTISOL_dn	49	B.6.8.3-01	Acceptable
	CEETOX_H295R_CORTISOL_up	50	B.6.8.3-01	Acceptable
	CEETOX_H295R_DOC_dn	51	B.6.8.3-01	Acceptable

Type of toxicity	Study	Study ID matrix	Reference	Acceptability
	CEETOX_H295R_DOC_up	52	B.6.8.3-01	Acceptable
	CEETOX_H295R_ESTRADIOL_dn	53	B.6.8.3-01	Acceptable
	CEETOX_H295R_ESTRADIOL_up	54	B.6.8.3-01	Acceptable
	CEETOX_H295R_ESTRONE_dn	55	B.6.8.3-01	Acceptable
	CEETOX_H295R_ESTRONE_up	56	B.6.8.3-01	Acceptable
	CEETOX_H295R_PROG_dn	57	B.6.8.3-01	Acceptable
	CEETOX_H295R_PROG_up	58	B.6.8.3-01	Acceptable
	CEETOX_H295R_TESTO_dn	59	B.6.8.3-01	Acceptable
	CEETOX_H295R_TESTO_up	60	B.6.8.3-01	Acceptable
	NVS_ADME_hCYP19A1	61	B.6.8.3-01	Acceptable
	TOX21_Aromatase_Inhibition	62	B.6.8.3-01	Acceptable

It should be noted that no information on the sodium salt of 2-phenylphenol (NaOPP) was included. Therefore, an evaluation of its endocrine disrupting properties was not addressed.

2. ED assessment for humans

2.1. ED assessment for T-modality

2.1.1 Have T-mediated parameters been sufficiently investigated?

	Sufficiently investigated
T-mediated parameters	Yes, based on availability of the following studies: OECD 409, 410, 452, 453 and US EPA 890.1450 and 890.1500

Some studies were conducted according to outdated versions of the test methods. Consequently, there are parameters related to endocrine activity that have not been measured in the repeated dose 90-day oral toxicity study in rodents (OECD TG 408), the developmental toxicity studies (OECD TG 414), the two-generation reproduction studies (OECD TG 416), and the carcinogenicity study (OECD 453, ID 13), as it is indicated in Table 2.10.2.1.1. In addition, in juvenile assays in rat (US EPA 890.1450 and 890.1500), T3 was not evaluated (in which it is an optional measurement).

Table 2.10.2.1.1: T-mediated parameters not measured

OECD TG 408 - T-mediated parameters not investigated
- Thyroid weight - T3 and/or T4 level - Thyroid stimulating hormone level (TSH) - Low-density lipoproteins (LDL) - High-density lipoproteins (HDL)
OECD TG 414 - T-mediated parameters not investigated
- T3 and/or T4 level (dams/rat) - Thyroid stimulating hormone level (TSH) (dams/rat) - Thyroid histopathology (dams/rat) - Thyroid weight (dams/rat)
OECD TG 416 - T-mediated parameters not investigated
- Follicular cell height (thyroid histopathology) - Thyroid histopathology (optional) - Thyroid weight

OECD TG 452 - T-mediated parameters not investigated
- Thyroid weight - Liver weight
US EPA 890.1450/1500 - T-mediated parameters not investigated
- T3

- OECD TG 409 (Thyroid weight and histopathology were measured)

- OECD TG 410 (Thyroid histopathology was measured)

- OECD TG 452 (Thyroid histopathology was measured)

- OECD TG 453 (Thyroid weight and histopathology were measured)

- US EPA 890.1450/1500 (Thyroid weight and histopathology, T4 and TSH were measured)

Thyroid weight was measured in study ID 5 (OECD 410), considered only as supporting information, in which dogs suffered emesis at all doses; in juvenile studies (US.EPA 890.1450 and 890.1500, studies ID 31 and 32, respectively), where the highest dose was above MTD and in study ID 10 (OECD 453). Regarding thyroid histopathological data, as it is present in the most of studies, overall, the available data are considered adequate for the assessment of T modality.

2.1.2 Lines of evidence for adverse effects and endocrine activity related to T-modality

Table 2.10.2.1.2: Lines of evidence for adverse effects and endocrine activity related to T-modality for humans

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
35	In vitro mechanistic	Thyroid receptor	human liver cell line	24 h	Uptake from the medium (in vitro)	>100	µM	No effect	no agonist	No T-mediated activity in vitro	No evidence for thyroid activity	T
36	In vitro mechanistic	Thyroid receptor	human THRa	1 h	Uptake from the medium (in vitro)	>50	µM	No effect	no antagonist			
37	In vitro mechanistic	Thyroid receptor	rat pituitary cell line	28 h	Uptake from the medium (in vitro)	>90	µM	No effect	no agonist			
38	In vitro mechanistic	Thyroid receptor	rat pituitary cell line	28 h	Uptake from the medium (in vitro)	>90	µM	No effect	no antagonist			
64	In vitro mechanistic	TSH receptor (in vitro)	human kidney cell line	0.5 h	Uptake from the medium (in vitro)	>90	µM	No effect	no agonist			
65	In vitro mechanistic	TSH receptor (in vitro)	human kidney cell line	0.5 h	Uptake from the medium (in vitro)	>90	µM	No effect	no antagonist			
66	In vitro mechanistic	TSH receptor (in vitro)	human kidney cell line	0.5 h	Uptake from the medium (in vitro)	>90	µM	No effect				
63	In vitro mechanistic	TRH receptor (in vitro)	rat TRHR	5 h	Uptake from the medium (in vitro)	>90	µM	No effect				
29	In vivo mechanisti	Adrenals weight (Hershberger)	rat	10 d	Oral	>1000	mg/kg bw/day	No effect		Effects on adrenal at high		

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	c									doses in antagonistic assay at the highest dose.		
30	In vivo mechanistic	Adrenals weight (Hershberger)	rat	10 d	Oral	1000	mg/kg bw/day	No effect	17% decrease (no statistically significant)			
29	In vivo mechanistic	Liver weight (Hershberger, considered T-mediated only in combination with other thyroid endpoints)	rat	10 d	Oral	>1000	mg/kg bw/day	No effect		No statistical changes in liver in Hershberger assays		
30	In vivo mechanistic	Liver weight (Hershberger, considered T-mediated only in combination with other thyroid endpoints)	rat	10 d	Oral	>1000	mg/kg bw/day	No effect	16% increase (no statistically significant)			
31	In vivo mechanistic	T3 and T4 level	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect	No change in T4. T3 not measured.	No evidence of consistent effects on T hormones in peripubertal assays. T4 was decreased in males at all doses with no further changes in thyroid or other hormones. Even at doses above MTD.		
32	In vivo mechanistic	T3 and T4 level	rat	PND 23-53	Oral	50	mg/kg bw/day	Decrease	T4: -15%; -23%; -22% at 50; 250; and 900 mg/kg/day, respectively; T4 of control group was above laboratory HCD. T3 not measured			
31	In vivo mechanistic	Thyroid-stimulating hormone level (TSH)	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
32	In vivo mechanisti	Thyroid-stimulating	rat	PND 23-53	Oral	>900	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	c	hormone level (TSH)										
5	EATS-mediated	Thyroid histopathology	dog	1 yr	Oral	>300	mg/kg bw/day	No effect		No evidence for thyroid adversity. Increased incidence of cysts in a dermal long-term study in females. No other thyroid effects were seen even at doses above MTD.	Overall, no evidence for thyroid adversity.	
6	EATS-mediated	Thyroid histopathology	dog	1 yr	Oral	>500	mg/kg bw/day	No effect				
8	EATS-mediated	Thyroid histopathology	mouse	4 wk	Dermal	>55.5	mg/kg bw/day	No effect				
10	EATS-mediated	Thyroid histopathology	rat	2 yr	Oral	>8000/>10'000	ppm	No effect				
12	EATS-mediated	Thyroid histopathology	mouse	2 yr	Oral	>1000	mg/kg bw/day	No effect				
13	EATS-mediated	Thyroid histopathology	mouse	102 wk	Dermal	55.5	other	Increase	Increased incidence of follicular cysts (20/46, 43%) in the thyroid gland of female mice dosed with 55.5 mg/0.1 mL compared with controls (6/47, 13%)			
31	EATS-mediated	Thyroid histopathology	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
32	EATS-mediated	Thyroid histopathology	rat	PND 23-53	Oral	>900	mg/kg bw/day	No effect				
5	EATS-mediated	Thyroid weight	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
10	EATS-mediated	Thyroid weight	rat	2 yr	Oral	>8000/>10'000	ppm	No effect				
31	EATS-mediated	Thyroid weight	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
32	EATS-mediated	Thyroid weight	rat	PND 23-53	Oral	>900	mg/kg bw/day	No effect				
3	Sensitive to, but not	Adrenals histopathology	rat	3 mo	Oral	>20'000	ppm	No effect		No consistent effects on		

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	diagnostic of, EATS									adrenal histopathology. Adrenal weight alterations were not correlated to histological changes except in the dermal 2-year study in mice. Increases in adrenal weight seem to occur in males and decreases in females.		
4	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	rat	13 wk	Oral	>25'000	ppm	No effect				
5	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
6	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	dog	1 yr	Oral	>500	mg/kg bw/day	No effect				
9	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	rat	2 yr	Oral	20'000	ppm	No effect	Not specified (at the highest dose)			
10	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	rat	2 yr	Oral	>8000/>10'000	ppm	No effect				
12	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	mouse	2 yr	Oral	>1000	mg/kg bw/day	No effect				
13	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	mouse	102 wk	Dermal	>55.5	other	Increase	Increased incidences of lipoid degeneration in the zona fasciculata of the adrenal gland in 1/49 vehicle control, 4/45 o-phenylphenol, male mice and			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									in 4/50 vehicle control, 24/47 o-phenylphenol female mice.			
31	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
32	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
4	Sensitive to, but not diagnostic of, EATS	Adrenals weight	rat	13 wk	Oral	25'000	ppm	Increase	+22%/+9.8% (m/f) increase in adrenal relative weight at the highest dose.			
5	Sensitive to, but not diagnostic of, EATS	Adrenals weight	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
9	Sensitive to, but not diagnostic of, EATS	Adrenals weight	rat	2 yr	Oral	20'000	ppm	No effect				
10	Sensitive to, but not diagnostic of, EATS	Adrenals weight	rat	2 yr	Oral	10'000	ppm	Decrease	Decrease in females adrenal weight at the dose of 647 mg/kg/day (10000 ppm) of 13.6%. No change in relative weight.			
12a	Sensitive to, but not diagnostic	Adrenals weight	mouse	2 yr	Oral	250	mg/kg bw/day	Increase	+16%; 18%; and 50% increase in			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	of, EATS								males in relative adrenal weight. Increase of 33% in adrenal absolute weight at the dose of 1000 mg/kg/day			
31	Sensitive to, but not diagnostic of, EATS	Adrenals weight	rat	PND 22-42	Oral	900	mg/kg bw/day	Decrease	-12.8% adjusted weight. Relative or unadjusted weight did not vary.			
32	Sensitive to, but not diagnostic of, EATS	Adrenals weight	rat	PND 23-53	Oral	250	mg/kg bw/day	Increase	increase of 16% in absolute weight at 900 mg/kg/day. Increase at the two highest doses in the adjusted weight (for PND23) of 9% and 11%, respectively.			
4	Sensitive to, but not diagnostic of, EATS	Brain weight	rat	13 wk	Oral	25'000	ppm	Change	Decrease of 5% in absolute brain weight and increase of 18% in relative brain weight at the dose of 25'000 ppm in males. 10% increase in relative brain weight in females at the highest dose.	Alterations in brain weight that could be associated to decreases in body weight.		

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
7	Sensitive to, but not diagnostic of, EATS	Brain weight	rat	3 wk	Dermal	>1000	mg/kg bw/day	No effect				
10	Sensitive to, but not diagnostic of, EATS	Brain weight	rat	2 yr	Oral	10’000	ppm	Increase	Increase in relative brain weight in males and females at the top dose of 402/647 mg/kg/day, respectively, of 7.8% and 18%.			
12	Sensitive to, but not diagnostic of, EATS	Brain weight	mouse	2 yr	Oral	500	mg/kg bw/day		Increases in relative brain weight in males and females of the top doses of 500 and 1000 mg/kg/day of 10% and 15% in males; and 15% and 23% in females, respectively			
14	Sensitive to, but not diagnostic of, EATS	Fertility (mammals)	rat	15/10 (P/F1) wk	Oral	>457	mg/kg bw/day	No effect		Fertility index was decreased in mouse. However, control groups of rat studies showed abnormally low fertility index. Therefore, this fact could be masking low fertilities in		
15	Sensitive to, but not diagnostic of, EATS	Fertility (mammals)	rat	10 wk	Oral	>458	mg/kg bw/day	Increase	Increased fertility index in one of the two F2 groups (31%). (This was attributed to the abnormally low control value).			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
18	Sensitive to, but not diagnostic of, EATS	Fertility (mammals)	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	No effect		treated groups. In addition, some deviation were noted in the determination of fertility.		
20	Sensitive to, but not diagnostic of, EATS	Fertility (mammals)	mouse	GD 7-15	Oral	2100	mg/kg bw/day	Increase	Fertility index: 14/21; 14/21 1450; 5/21; at the doses of 1740; and 2100 mg/kg/day, respectively. In control group 20/21			
14	Sensitive to, but not diagnostic of, EATS	Gestation length	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect		No effects on gestation length were observed.		
15	Sensitive to, but not diagnostic of, EATS	Gestation length	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
16	Sensitive to, but not diagnostic of, EATS	Litter size	rat	GD 6-15	Oral	>700	mg/kg bw/day	No effect		No effects observed in litter size.		
18	Sensitive to, but not diagnostic of, EATS	Litter size	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	No effect				
19	Sensitive to, but not diagnostic of, EATS	Litter size	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Litter size	mouse	GD 7-15	Oral	>2100	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
18	Sensitive to, but not diagnostic of, EATS	Litter viability	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	No effect		No effects observed in litter viability		
19	Sensitive to, but not diagnostic of, EATS	Litter viability	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Litter viability	mouse	GD 7-15	Oral	>2100	mg/kg bw/day	No effect	There was no significant difference and no dose dependence in respect of quantity.			
15	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	rat	10 wk	Oral	457	mg/kg bw/day	Decrease	At the dose of 458 mg/kg/day, decrease at day 21, in F1 pups' weights (12% and 10% in both groups), and in F2 at days 14 (5.7% and 4%) and at day 21 (10.6% and 12%).	Decreases in mouse and rat litter/pup weight in prenatal and 2 generation studies. Decreases in maternal body weight gain were observed.		
16	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	rat	GD 6-15	Oral	>700	mg/kg bw/day	No effect				
17	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	rat	GD 6-15	Oral	600	mg/kg bw/day	Decrease	6% decrease in males and 8.5% decrease in females at the dose of 600 mg/kg/day. 27% decrease in			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									males and 27% decrease in females at the dose of 1200 mg/kg/day.			
19	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	mouse	GD 7-15	Oral	1450	mg/kg bw/day	Decrease	Body weight of the live foetuses of both sexes was significantly reduced and a retardation of development must be assumed.			
16	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	rat	GD 6-15	Oral	>700	mg/kg bw/day	No effect		Decreased implantations in a developmental study in rat. However, this effect may be disregarded due to methodological deficiencies, as explained in EAS WoE section.		
17	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	rat	GD 6-15	Oral	>1200	mg/kg bw/day	Decrease	At the dose of 1200 mg/kg/day, 8 vs 11.5 in control group, only one litter at 1200 mg/kg bw/day			
18	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	No effect				
19	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
20	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	mouse	GD 7-15	Oral	>2100	mg/kg bw/day	No effect				
14	Sensitive to, but not diagnostic of, EATS	Number of live births	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect		No significant effects on live births were observed		
15	Sensitive to, but not diagnostic of, EATS	Number of live births	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
16	Sensitive to, but not diagnostic of, EATS	Number of live births	rat	GD 6-15	Oral	>700	mg/kg bw/day	No effect				
17	Sensitive to, but not diagnostic of, EATS	Number of live births	rat	GD 6-15	Oral	1200	mg/kg bw/day	Decrease	At the dose of 1200 mg/kg/day, 8 vs 11.5 in control group, only one litter at 1200 mg/kg bw/day			
5	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	dog	1 yr	Oral	>300	mg/kg bw/day	No effect		Pituitary histopathology was not significantly altered. Pituitary weight was decreased at the highest dose in the pubertal rat assays. IN males the decrease was in absolute and adjusted		
10	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	rat	2 yr	Oral	>8000/>10'000	ppm	No effect				
12	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	mouse	2 yr	Oral	>1000	mg/kg bw/day	No effect				
13	Sensitive to, but not diagnostic	Pituitary histopathology	mouse	102 wk	Dermal	>55.5	other	No effect				

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	of, EATS									weight, and in females in the relative weight.		
14	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	rat	15/10 (P/F1) wk	Oral	>457	mg/kg bw/day	No effect				
15	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
31	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
32	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	rat	PND 22-42	Oral	900	mg/kg bw/day	Increase	1 animal presented pale pituitary at the highest dose			
5	Sensitive to, but not diagnostic of, EATS	Pituitary weight	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
31	Sensitive to, but not diagnostic of, EATS	Pituitary weight	rat	PND 22-42	Oral	900	mg/kg bw/day	Decrease	9.8% less relative weight. Adjusted and unadjusted weight did not statistically vary.			
32	Sensitive to, but not diagnostic of, EATS	Pituitary weight	rat	PND 23-53	Oral	900	mg/kg bw/day	Decrease	Decrease in absolute and adjusted weight (PND23) at the highest dose (-15% and -11%, respectively)			
17	Sensitive to, but not	Post implantation loss	rat	GD 6-15	Oral	600	mg/kg bw/day	Increase	At the dose of 600 mg/kg/day	Some increases in post		

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	diagnostic of, EATS								25.7% vs. 13.9% in the control group. 38,5% in the 1200 mg/kg/day.	implantation loss were observed. As explained WoE section of EAS modalities, deviations in the test methods may be minimising their incidence.		
18	Sensitive to, but not diagnostic of, EATS	Post implantation loss	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	No effect				
19	Sensitive to, but not diagnostic of, EATS	Post implantation loss	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Post implantation loss	mouse	GD 7-15	Oral	>2100	mg/kg bw/day	No effect				
16	Sensitive to, but not diagnostic of, EATS	Pre implantation loss	rat	GD 6-15	Oral	>700	mg/kg bw/day	No effect				
18	Sensitive to, but not diagnostic of, EATS	Pre implantation loss	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	No effect				
19	Sensitive to, but not diagnostic of, EATS	Pre implantation loss	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Pre implantation loss	mouse	GD 7-15	Oral	1450	mg/kg bw/day	Increase				
16	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	rat	GD 6-15	Oral	700	mg/kg bw/day	Change	delayed ossification of skull, pinpoint holes in the	Increased incidence of anomalies was noted in		

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									occipital or interparietal plates in the skull, and skull bone island (outside HCD) delayed ossification of sternebrae (inside HCD)	rodents.		
17	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	rat	GD 6-15	Oral	300	mg/kg bw/day	Increase	Of the foetuses from 300 or 600 mg/kg group, only 1 or 2 showed concurrent occurrence of anomalies such as cranial or sacral meningocele and diaphragmatic hernia. However, the anomalies were too low in their incidences to be analysed by this study whether they were caused by OPP or not. A decrease in the maternal food-intake during the period of the treatment			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									might contribute to the occurrence of the anomalies. Foetuses survived their maternal treatment with 1200 mg/kg of OPP were free from anomalies.			
19	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	mouse	GD 7-15	Oral	1450	mg/kg bw/day	Change	there was a tendency, though not a significant one, for the number of cervical ribs to increase in a manner dependent on the dose.			
14	Sensitive to, but not diagnostic of, EATS	Pup survival index	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect		No effects on pup survival index		
15	Sensitive to, but not diagnostic of, EATS	Pup survival index	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
14	Sensitive to, but not diagnostic of, EATS	Sex ratio	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect		No alterations on sex ratio were observed.		

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
15	Sensitive to, but not diagnostic of, EATS	Sex ratio	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
16	Sensitive to, but not diagnostic of, EATS	Sex ratio	rat	GD 6-15	Oral	>700	mg/kg bw/day	No effect				
19	Sensitive to, but not diagnostic of, EATS	Sex ratio	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Sex ratio	mouse	GD 7-15	Oral	>2100	mg/kg bw/day	No effect				
14	Sensitive to, but not diagnostic of, EATS	Time to mating	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect				
15	Sensitive to, but not diagnostic of, EATS	Time to mating	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
4	Target organ toxicity	Kidney histopathology	rat	13 wk	Oral	25'000	ppm	Change	Inflammation in kidney at the highest dose	Decreases in absolute kidney weight, mainly in long term studies. Increases in histopathological alterations mainly at high doses.	Overall evidence of effects in kidney and liver.	
6	Target organ toxicity	Kidney histopathology	dog	1 yr	Oral	>500	mg/kg bw/day	No effect				
7	Target organ toxicity	Kidney histopathology	rat	3 wk	Dermal	>1000	mg/kg bw/day	No effect				
9	Target organ toxicity	Kidney histopathology	rat	2 yr	Oral	20'000	ppm	Increase	Extensive renal damage, characterised by tubular dilatation with			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									varying degrees of acute and chronic inflammation			
10	Target organ toxicity	Kidney histopathology	rat	2 yr	Oral	10000	ppm	Induction	7 females of the dose of 647 mg/kg/day (10'000 ppm) vs 0 in control group presented pitted zones and 8 vs 1 presented abnormal texture. Increased incidence of renal infarct (29 vs 3) in females; hyperolasia (30 vs 3) in females; cyst in males (17 vs 4) and females (37 vs 14); acute inflammation (M: 7, 11, 3, 5; F: 2, 0, 0, 11 *) and in the incidence of mineralization within the tubules of the renal papilla was noted (F:0,0,2,12*) in 10,000 ppm females.			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
11	Target organ toxicity	Kidney histopathology	rat	91 week	Oral	12’500 ppm (531 mg/kg/day)	ppm	Induction	Moderate to severe nephritic lesions appeared in 3/24 (13%) of the 1.25% group and 23/23 (100%) of the 2.5% group. The incidence of this lesion was significantly higher in the 2.5% group than in the controls. Among these lesions, moderate to severe pyelonephritis with papillary destruction were found in 1/3 (33%) of the 1.25% and 15/23 (65%) of the 2.5% groups, and the other lesion was interstitial nephritis.			
12	Target organ toxicity	Kidney histopathology	mouse	2 yr	Oral	250	mg/kg bw/day	Increase	A dose-related decrease in the incidence of microvacuolation in the kidney			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									tubules of male mice was observed at all dose levels.			
14	Target organ toxicity	Kidney histopathology	rat	15/10 (P/F1) wk	Oral	457	mg/kg bw/day	Decrease	In P males at the highest dose, increase in calculus (13 vs 3) and hemorrhage (6 vs 0)			
15	Target organ toxicity	Kidney histopathology	rat	10 wk	Oral	458	mg/kg bw/day	Increase	P and F1 males did appear to have a greater number of animals with numerous background lesions, multiple lesions with severity grades of slight to marked, and/or lesions such as chronic active inflammation and debris in the renal pelvis that were noted only in the high-dose level males (no statistically significant)			
18	Target organ toxicity	Kidney histopathology	rabbit	GD 7-19	Oral	500	mg/kg bw/day	Change	dose dependent alterations			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
19	Target organ toxicity	Kidney histopathology	rabbit	GD 7-19	Oral	250	mg/kg bw/day	Change	Treatment-related effects on the kidneys were observed in 10 of 24 (42%) rabbits at 250 mg/kg/day. The kidneys had tubular degeneration, focal to multifocal in distribution, slight to moderate in degree, accompanied by inflammation that was focal to multifocal in distribution, and slight in degree.			
31	Target organ toxicity	Kidney histopathology	rat	PND 22-42	Oral	900	mg/kg bw/day	Induction	very slight or slight focal or multifocal dilation of the renal tubule (2 vs 11 in the control group and 900 mg/kg/day, respectively), sometimes accompanied by degeneration and necrosis (0 vs 2); slight hyperplasia of			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									the epithelium lining the papilla and very slight hypertrophy of the epithelial cells (0 vs 1 in the control and 900 mg/kg/day, respectively) lining the collecting duct.			
32	Target organ toxicity	Kidney histopathology	rat	PND 23-53	Oral	900	mg/kg bw/day	Increase	Control vs 900 mg/kg/day group effects: Dilatation, tubule, focal/multifocal –Very slight or Slight (4 vs 12, respectively); Hypertrophy, collecting duct, epithelium, focal/multifocal –Very slight (0 vs 5, respectively); hyperplasia, epithelium, papilla, unilateral or bilateral, multifocal – Very slight (0 vs 2)			
1	Target organ	Kidney weight	rabbit	13 d	Oral	100	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	toxicity											
4	Target organ toxicity	Kidney weight	rat	13 wk	Oral	6250	ppm	Increase	Increases of 4.3%; 5.7%; and 25% in the kidney relative weight in males at the doses of 6250, 12'500, 25'000 ppm, respectively. No changes in absolute weight. In females, increase of 15% in the relative kidney weight at the highest dose.			
5	Target organ toxicity	Kidney weight	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
6	Target organ toxicity	Kidney weight	dog	1 yr	Oral	500	mg/kg bw/day	Increase	Slight increase in kidney weight at the top dose of 500 mg/kg/day (not specified)			
7	Target organ toxicity	Kidney weight	rat	3 wk	Dermal	>1000	mg/kg bw/day	No effect				
10	Target organ toxicity	Kidney weight	rat	2 yr	Oral	4000	ppm	Decrease	Decreased kidney weight in females at the doses of 8% and 11% at the doses of 248 and 647			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									mg/kg/day, respectively (4000 and 10000 ppm). No change in relative weight.			
12	Target organ toxicity	Kidney weight	mouse	2 yr	Oral	500	mg/kg bw/day		Decrease in males in absolute kidney weight of 7% and 14% at the doses of 500 and 1000 mg/kg/day, respectively. And increases in relative kidney weight in females 17% and 20% at the highest doses.			
14	Target organ toxicity	Kidney weight	rat	15/10 (P/F1) wk	Oral	457	mg/kg bw/day	Increase	At the highest dose of 457 mg/kg/day, increase in P and F1 relative kidney weights in males (8% and 11%, respectively). Decrease in absolute kidney weights in P females (9.4%).			
18	Target organ toxicity	Kidney weight	rabbit	GD 7-19	Oral	500	mg/kg bw/day	Increase	Increased relative weight (34%) at the dose of 500			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									mg/kg/day (the highest dose at which this parameter was measured).			
19	Target organ toxicity	Kidney weight	rabbit	GD 7-19	Oral	>250	mg/kg bw/day	No effect				
31	Target organ toxicity	Kidney weight	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
32	Target organ toxicity	Kidney weight	rat	PND 23-53	Oral	>900	mg/kg bw/day	No effect				
3	Target organ toxicity	Liver histopathology	rat	3 mo	Oral	>20'000	ppm	No effect		Alterations in liver weight in rodents in studies longer than 90 days except in one. Dams seem to present a light tendency to have a decrease in liver weight is observed in the developmental studies and in F1 animals of one two generation study. Histological findings were observed in two long term		
4	Target organ toxicity	Liver histopathology	rat	13 wk	Oral	25’000	ppm	No effect				
5	Target organ toxicity	Liver histopathology	dog	1 yr	Oral	300	mg/kg/day	No effect				
6	Target organ toxicity	Liver histopathology	dog	1 yr	Oral	>500	mg/kg bw/day	No effect				
7	Target organ toxicity	Liver histopathology	rat	3 wk	Dermal	>1000	mg/kg bw/day	No effect	Not specified (at the highest dose)			
8	Target organ toxicity	Liver histopathology	mouse	4 wk	Dermal	55.5 mg/0.1 mL	mg/mL	No effect				
9	Target organ toxicity	Liver histopathology	rat	2 yr	Oral	20'000	ppm	Increase	Not specified (at the highest dose)			
10	Target organ	Liver histopathology	rat	2 yr	Oral	>10000 (402 mg/kg/day	ppm	No effect				

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	toxicity					males/ 647 mg/kg/day for females)				studies at the highest doses and in the only prenatal developmental study that it was measured.		
12	Target organ toxicity	Liver histopathology	mouse	2 yr	Oral	500	mg/kg bw/day	Increase	Gross necropsy observations in the middle and high dose males, suggested a slight increase in the number of mice with a liver mass/nodule. A dose-related increased in the incidence of "accentuated lobular pattern" was observed at all dose levels in both sexes. Incidence of male mice with hepatocellular adenoma was statistically significantly increased in the middle and high dose groups.			
14	Target organ toxicity	Liver histopathology	rat	15/10 wk (P/F1) wk	Oral	>457	mg/kg bw/day	No effect				
15	Target organ toxicity	Liver histopathology	rat	10 wk	Oral	458	mg/kg bw/day	Increase	At the dose of 458 mg/kg/day, 2 F1 males showed			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									malignant lymphoma and 1 male showed necrosis (not statistically significant)			
18	Target organ toxicity	Liver histopathology	rabbit	GD 7-19	Oral	250	mg/kg bw/day	Increase	1; 2; 5 animals presented autolysis vs 0 in the control group.			
1c	Target organ toxicity	Liver weight	rabbit	13 d	Oral	>1000	mg/kg bw/day	No effect				
3	Target organ toxicity	Liver weight	rat	3 mo	Oral	10’000	ppm	Increase	Increases in liver weight at the doses of 10'000 and 20'000 ppm			
4	Target organ toxicity	Liver weight	rat	13 wk	Oral	3130	ppm	Increase	Increases in males of 7%; 7.3%; 11%; 20% in relative liver weight at the doses of 3130, 6250, 12'500, 25'000, respectively. No changes in absolute liver weights. In females relative increases of 13%; and 33% at the two highest doses, respectively. Increase of 15%			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									at the highest dose in absolute liver weight in females.			
5	Target organ toxicity	Liver weight	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
7	Target organ toxicity	Liver weight	rat	3 wk	Dermal	>1000	mg/kg bw/day	No effect				
9	Target organ toxicity	Liver weight	rat	2 yr	Oral	>20’000	ppm	No effect				
10	Target organ toxicity	Liver weight	rat	2 yr	Oral	4000 ppm (248 mg/kg/day)	ppm	Decrease	Decreased liver weight in females 9.5% and 12.5% at the doses of 248 and 647 mg/kg/day, respectively (4000 and 10000 ppm). No change in relative weight.			
12	Target organ toxicity	Liver weight	mouse	2 yr	Oral	500	mg/kg bw/day	Increase	Increase in females in absolute liver weight of 36% and 23% at the doses of 500 and 1000 mg/kg/day, respectively. Increase of liver relative weight 16%; 56%; and 46% at 250, 500			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									and 1000 mg/kg/day, respectively.			
14	Target organ toxicity	Liver weight	rat	15/10 (P/F1) wk	Oral	457	mg/kg bw/day	Decrease	Decreased absolute liver weight (13.4%) in F1 females at the dose of 457 mg/kg/day			
16a	Target organ toxicity	Liver weight	rat	GD 6-15	Oral	700	mg/kg bw/day	Decrease	At the dose of 700 mg/kg/day, absolute liver weight decreased 17%. Relative weight did not change.			
18	Target organ toxicity	Liver weight	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	No effect				
19	Target organ toxicity	Liver weight	rabbit	GD 7-19	Oral	>250	mg/kg bw/day	No effect				
31	Target organ toxicity	Liver weight	rat	PND 22-42	Oral	900	mg/kg bw/day	Increase	+9.3% relative to BW. Adjusted and unadjusted weight did not vary.			
32	Target organ toxicity	Liver weight	rat	PND 23-53	Oral	250	mg/kg bw/day	Increase	+8% and +21% in relative liver weight at the doses of 250 and 900 mg/kg/day; increase at the highest dose of adjusted (for PND 23) weight			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									of 10%. No difference in unadjusted weight			
1a	Systemic toxicity	Body weight	rabbit	13 d	Oral	100	mg/kg bw/day	Decrease	Decreased BW (24%) at the highest dose of 1000 mg/kg/day.	Signs of systemic toxicity occurred at high doses, which included mainly clinical signs, effects on body weight, food consumption, haematology, and clinical chemistry; these signs are related to general toxicity of higher doses as generally seen in toxicology studies. However, a case by case approach may be done, as toxic adverse effects were not observed in all studies.	Overall evidence of systemic toxicity.	
2	Systemic toxicity	Body weight	dog	4 wk	Oral	300	mg/kg bw/day	Decrease	decreased BW gain in females at the dose of 300 mg/kg/day			
3	Systemic toxicity	Body weight	rat	3 mo	Oral	20'000	ppm	Increase	Slight decrease in gain weight at the highest dose group.			
4	Systemic toxicity	Body weight	rat	13 wk	Oral	25'000	ppm	Decrease	-22%/-11% (m/f) decrease at the highest dose.			
5	Systemic toxicity	Body weight	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
7	Systemic toxicity	Body weight	rat	3 wk	Dermal	>1000	mg/kg bw/day	No effect				
8	Systemic toxicity	Body weight	mouse	4 wk	Dermal	>55.5	mg/0.1mL	No effect				
9	Systemic toxicity	Body weight	rat	2 yr	Oral	>20'000	ppm	Decrease				
10	Systemic toxicity	Body weight	rat	2 yr	Oral	8000/10'000	ppm	Decrease	-11% decrease in body weight gain at the highest dose in males and females. Decrease of 9% and 7.7% in the body weight in			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									males and females, respectively.			
11	Systemic toxicity	Body weight	rat	91 wk	Oral	12'500 ppm; 531 mg/kg/d	ppm	Decrease	-12%			
12	Systemic toxicity	Body weight	mouse	2 yr	Oral	500	mg/kg bw/day	Decrease	27% decrease in body weight gain in males at the highest dose; and 25% and 38% in females at the two highest doses. Decrease in body weight of 12.8% in males of the 1000 mg/kg/day; and decrease of 13% and 20% in the females of the two highest doses.			
13	Systemic toxicity	Body weight	mouse	102 wk	Dermal	55,5	other	Decrease				
14a	Systemic toxicity	Body weight	rat	15/10 (P/F1) wk	Oral	457	mg/kg bw/day	Decrease	Decrease in body weights at the highest dose of 457 mg/kg/day in pre mating periods in P males (7%) and F1 in males (12.2%) and females (10.7%).			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									Decrease BW gain in P animals (23% and 24.4% in males and females, respectively) and F1 (13% and 20% in males and females, respectively). Decreases in body weight in females in GD0 (7% and 10% in the two control F0 dams; and 8% and 9% in the two control F1 dams); GD6 (4% and 8% in the two control F0 dams; and 3% and 7% in the two control F1 dams); and GD13 (9% and 8% in the two control F1 dams). Decreases in body weight in females during in LD4 and LD7 in one of the F0 control groups (7% and			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									6%, respectively); and decreases in F1 LD0 controls (6% and 8% in both controls); LD4 (10% and 11%); LD7 (6% and 8%) and LD14 (8% in the second control group). The second F1 control group also showed and increase BWG during lactating period of 120%.			
14b	Systemic toxicity	Body weight	rat	15/10 (P/F1) wk	Oral	457	mg/kg bw/day	Decrease	Decrease in body weights at the highest dose of 457 mg/kg/day in F1B litters at day 21 (18%); F2B litters (12%); and F2A litters in days 14 and 21 (7% and 12%, respectively).			
15	Systemic toxicity	Body weight	rat	10 wk	Oral	458	mg/kg bw/day	Decrease	At the highest dose of 458 mg/kg/day, decreased body weight			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									throughout the experiment F1 females (9%), and F1 males (11%). Decrease in P females (7%) from day 21. During gestation (5-7%) and lactating days (5-7%) decreases at al measured days in F0 and F1.			
16	Systemic toxicity	Body weight	rat	GD 6-15	Oral	700	mg/kg bw/day	Decrease	At the dose of 700 mg/kg/day, decrased weight on GD 10 of 5.6% and on GD 16 of 5.7%. Body weight gain was decreased between days 6-9 (35%)			
17	Systemic toxicity	Body weight	rat	GD 6-15	Oral	300	mg/kg bw/day	Decrease	At the dose of 300 mg/kg/day, decreases in BWG at GD9: 17%; at GD 12: 18%; at GD 15: 28%; at GD 20: 20%. At the dose of 600 mg/kg/day, decreases in			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									BWG at GD9: 60%; at GD 12: 51%; at GD 15: 62% of controls; at GD 20: 46% (BW not measured).			
18	Systemic toxicity	Body weight	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	Decrease	At the dose of 750 mg/kg/day reduced body weight on GD13 (19%) and GD16 (29%).			
19	Systemic toxicity	Body weight	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Systemic toxicity	Body weight	mouse	GD 7-15	Oral	1740	mg/kg bw/day	Decrease	Decreased body weight at all doses both in males (4%; 5%; 20%, at 1450; 1740; and 2100 mg/kg/day respectively) and females (8%; 4%; 20%, at 1450; 1740; and 2100 mg/kg/day respectively).			
28c	Systemic toxicity	Body weight	rat	PND 19-22	Oral	1000	mg/kg bw/day	Decrease	BW gain: 75% of controls at day 4. No difference in BW.			
29	Systemic toxicity	Body weight	rat	10 d	Oral	1000	mg/kg bw/day	Decrease	BW gain: 59% of controls (no statistically			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									significant)			
30	Systemic toxicity	Body weight	rat	10 d	Oral	1000	mg/kg bw/day	Decrease	BW gain: 73% of controls (no statistically significant)			
31	Systemic toxicity	Body weight	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect	BW gain: -12.9% between PND 22-35 (no statistically significant); no difference at the end of the experiment (PND42). No difference in BW.			
32	Systemic toxicity	Body weight	rat	PND 23-53	Oral	900	mg/kg bw/day	Decrease	-11.6% in body weight and -12,6% in body weight gain in the highest dose group.			
3	Systemic toxicity	Clinical chemistry and haematology	rat	3 mo	Oral	>20'000	ppm	No effect	Normal BUN levels			
4	Systemic toxicity	Clinical chemistry and haematology	rat	13 wk	Oral	12'500	ppm	Increase	1.25% Females: significantly reduced Hb and MCH; 2.5% Females: significantly reduced Hb and MCH. Males: significantly reduced RBC, Hb and MCHC.			
5	Systemic toxicity	Clinical chemistry and haematology	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
6	Systemic toxicity	Clinical chemistry and haematology	dog	1 yr	Oral	500	mg/kg bw/day	No effect				
7	Systemic toxicity	Clinical chemistry and haematology	rat	3 wk	Dermal	>1000	mg/kg bw/day	No effect				
10	Systemic toxicity	Clinical chemistry and haematology	rat	2 yr	Oral	>8000/10'000	ppm	Change	Increase in BUN (27%) in females at the highest dose and decrease of triglycerides (56%). In males increase in ALP (35% at the highest dose). In males decrease in triglycerides (44% and 61%, respectively at the two highest doses) and cholesterol (36% and 51% at the two highest doses). Decrease of proteins in urine in males (23% and 75% at the two highest doses, respectively) and in females (50% and 86% at the two highest doses, respectively). However, no			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									confirmation of OPP-induced clinical chemistry or hematology changes in this study in either sex at any dose tested.			
12	Systemic toxicity	Clinical chemistry and haematology	mouse	2 yr	Oral	500	mg/kg bw/day	No effect				
31	Systemic toxicity	Clinical chemistry and haematology	rat	PND 22-42	Oral	900	mg/kg bw/day	Induction	Alanine aminotransferase (+102%), blood urea nitrogen (+23%), and phosphorus (+14%) levels were increased at 900 mg/kg/day			
32	Systemic toxicity	Clinical chemistry and haematology	rat	PND 23-53	Oral	900	mg/kg bw/day	Increase	Animals given 900 mg/kg/day had statistically-identified increase (27%) in BUN concentration; increases in serum ALT (95%) and AST (32%) activities.			
5	Systemic toxicity	Clinical signs	dog	1 yr	Oral	300	mg/kg bw/day	Increase	emesis after treatment at the dose of 300			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									mg/kg/day			
18	Systemic toxicity	Clinical signs	rabbit	GD 7-19	Oral	250	mg/kg bw/day	Increase	soft faeces and perineal soiling			
19	Systemic toxicity	Clinical signs	rabbit	GD 7-19	Oral	250	mg/kg bw/day	Increase	decreased faeces, decreased activity, perineal soiling, blood in pan			
28b	Systemic toxicity	Clinical signs	rat	PND 19-22	Oral	>1000	mg/kg bw/day	No effect				
29	Systemic toxicity	Clinical signs	rat	10 d	Oral	1000	mg/kg bw/day	Change	In the highest dose animals, decreased activity, noisy respiration, clear or red perioral soiling, perineal soiling (urine and/or feces), and soft feces were observed. In the last period (days 7-11), 2 animals showed noisy respiration and a thir animal had perioral (clear) soiling.			
30	Systemic toxicity	Clinical signs	rat	10 d	Oral	1000	mg/kg bw/day	Change	In the highest dose group, one animal (excluded from the study) showed			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									decreased activity; noisy respiration; perioral (clear) soiling; slow respiration; labored respiration; perineal (urine) soiling. Another animal showed perioral (clear) soiling; slow respiration; decreased activity; perineal (urine) soiling; perinasal (red) soiling. And a third animal showed Noisy respiration; perioral (clear) soiling.			
32	Systemic toxicity	Clinical signs	rat	PND 23-53	Oral	>900	mg/kg bw/day	No effect				
4	Systemic toxicity	Food consumption	rat	13 wk	Oral	25'000	ppm	Decrease				
11	Systemic toxicity	Food consumption	rat	91 wk	Oral	25'000 ppm; 1140 mg/kg/d	ppm	Decrease	At the highest dose, significantly reduced food intake (g/rat). Increased relative food intake (g/kg bw/day)			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
29	Systemic toxicity	Food consumption	rat	10 d	Oral	1000	mg/kg bw/day	Decrease	Day 4-7: 58% of controls. In the final period (7-11) no difference was observed.			
30	Systemic toxicity	Food consumption	rat	10 d	Oral	1000	mg/kg bw/day	Decrease	Day 4-7: 58% of controls (no statistically different in the last period 7-11)			
4	Systemic toxicity	Mortality	rat	13 wk	Oral	25'000	ppm	Increase	2 males and 1 female of the highest dose group died			
10	Systemic toxicity	Mortality	rat	2 yr	Oral	8000/10'000	ppm	Increase	Increase in mortality of the highest dose group (402 mg/kg/day) in males: 19 in control vs 24 in this group.			
11	Systemic toxicity	Mortality	rat	91 wk	Oral	12'500 ppm; 531 mg/kg/d	ppm	Increase	Survival: 71% vs 96% (highest dose vs control)			
17	Systemic toxicity	Mortality	rat	GD 6-15	Oral	1200	mg/kg bw/day	Increase	10/11 dams died after 3-9 days of treatment at the dose of 1200 mg/kg/day			
18	Systemic toxicity	Mortality	rabbit	GD 7-19	Oral	500	mg/kg bw/day	Increase	2/7 at 500 mg/kg/d and 6/7 at 750 mg/kg/d (deposition of test material in			

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									lungs). Due to the high rate of mortality, only one litter containing two embryos undergoing resorption was available in the 750 mg/kg group.			
19	Systemic toxicity	Mortality	rabbit	GD 7-19	Oral	250	mg/kg bw/day	Increase	At the 250 mg/kg/day group, 4/24 treatment-related deaths.			
20	Systemic toxicity	Mortality	mouse	GD 7-15	Oral	1450	mg/kg bw/day	Increase	4; 5; and 16 death females in the groups of 1450; 1740; and 2100 mg/kg/day, respectively.			
28a	Systemic toxicity	Mortality	rat	PND 19-22	Oral	>1000	mg/kg bw/day	No effect				
29	Systemic toxicity	Mortality	rat	10 d	Oral	>1000	mg/kg bw/day	No effect				
30	Systemic toxicity	Mortality	rat	10 d	Oral	>1000	mg/kg bw/day	No effect				
32	Systemic toxicity	Mortality	rat	PND 23-53	Oral	>900	mg/kg bw/day	No effect				

2.1.2.1 Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

Table 2.10.2.1.2.1/1: WoE for T-mediated adversity

- Thyroid histological changes were only observed in the 2-year dermal study in mice (ID 13), at a dose of 55.5 mg/0.1 mL (dermal carcinogenicity study); however, only females were affected.
- In study ID 13, liver histopathology and weight were not measured. Body weight was only altered in males. No other parameters which could indicate systemic toxicity were analysed.
- No histopathological alterations in thyroid were seen in any other study. In dog 1-year (ID 5), up to a dose of 300 mg/kg/day (emesis was observed and treatment was given only 5 times per week) and in ID 6, up to 500 mg/kg/day; in mouse 4-weeks (ID 8) up to a dose of 55.5 mg/0.1 mL); and ID 12, 102 weeks (combined chronic toxicity and carcinogenicity), at doses up to 1000 mg/kg/day; in rat, in study ID 10, 2 years (combined chronic toxicity and carcinogenicity) up to 402 (m) and 647 (f) mg/kg/day; and in studies ID 31 and 32, PND 22-42 and PND 23-53, respectively, up to a dose of 900 mg/kg/day.
- Thyroid weight showed no variations in the studies in which it was measured: 1-year dog, ID 5 (emesis and treatment only 5 days per week); rat 2 years, ID 10; rat PND 22-42 and PND 23-53, IDs 31 and 32, respectively.
- Studies ID 5, ID 10 (combined chronic toxicity and carcinogenicity), ID 31 and ID 32 are studies where thyroid weight and histopathology were both measured, and where no significant alteration were seen, including doses which induced systemic toxicity and above MTD (except in the ID 5 dog study, in which emesis was observed at all doses and treatment was done only 5 days per week).
- Regarding the above-mentioned studies were both thyroid weight and histopathology were measured, liver weight was altered in 2-year rat study (ID 10), where it was decreased in females at the doses of 248 and 647 mg/kg/day, respectively (4000 and 10000 ppm) and in pubertal studies ID 31 and 32, where increases in liver weight were seen. In this rat 2-year study (ID 10), liver histopathology was not altered at doses up to 402 mg/kg/day in males and in females at 647 mg/kg/day (ID 10). In the study in dogs ID 5, no alterations in liver were observed.
- In study ID 13, in which thyroid histopathology was altered, no liver parameters were measured, nevertheless, effects in liver histopathology were observed in the 2-year study in mouse (ID 12) from the dose of 250 mg/kg/day in both males and females; in the two generation rat study (ID 15) at the dose of 458 mg/kg/day in males; and in one of the prenatal developmental studies from the dose of 250 mg/kg/day (ID 18), where animals presented autolysis in a dose-dependent manner.
  In addition, liver weight was altered in any way in all studies except in ID 1 (rabbit, 13 days, oral); ID 5 (dog, 1 year, oral); ID 7 (rat, 3 weeks, dermal); ID 9 (publication of rat chronic oral study); ID 18 and 19 (prenatal developmental studies in rabbit).
- Alterations in kidney histopathology (ID 4, 9, 10, 12, 15, 18, 19, 31, 32, from doses of 250 mg/kg/day), and in kidney weight (ID 4, 6, 10, 12, 14, 18, 19) were also observed.
- Alterations in liver histopathology were observed in studies ID 12, 15, 18 from doses of 250 mg/kg/day, and in liver weight in studies ID 3, 4, 10, 12, 14, 16, 31 and 32.

Table 2.10.2.1.2.1/2: WoE for T-mediated endocrine activity

- TSH was analyzed in studies ID 31 and 32 (juvenile female/male rat), showing no variations in the parameter neither in females nor in males.
- In juvenile/peripubertal male rats OPP displayed effects on T4 (decreases of -15%; -23%; -22% at the

doses of 50; 250; and 900 mg/kg/day, respectively) T4 of control group was above laboratory HCD. In addition T4 was not altered in females. T3 was not measured and thyroid follicular cell hypertrophy and colloid changes were not observed.

- ToxCast and Tox21 thyroid hormone assays were negative for OPP.
- Throid receptor, TSH receptor, TRH receptor were not altered in *in vitro* mechanistic studies.

No effects were observed in thyroid weight or histopathology except in the 2 year repeated dose dermal toxicity study in mouse (ID: 13), where an increased incidence of follicular cysts (20/46, 43%) in the thyroid gland of female mice dosed with 55.5 mg/0.1 mL of OPP compared with controls (6/47, 13%) was found.

However, it is considered that this effect does not implicate T-mediated adversity based upon following argumentation:

- In males the effect was not observed.
- Although in this study the thyroid weight has not been measured, there are no consistent effects on thyroid weight in other studies.
- This effect was only observed in the 2-year repeated dose dermal toxicity study, in a single species (mouse), and no effects on thyroid weight or histopathology were observed in rat or dog studies over significantly long dosing periods. As such, the thyroid was not a target organ in the same species at higher doses, via other routes of exposition or similar or shorter duration of treatment.
- There was no consistency in the effects in mouse, as no adverse effect on thyroid histopathology was observed in this animal in a 2-year oral exposure experiment.
- In studies in which both thyroid weight and histopathology were measured, (ID 5; ID 10; ID 31; ID 32), no effects were observed at doses up to 900 mg/kg/day, including a rat 2 year combined chronic toxicity/carcinogenicity study, in which males were treated with 39; 200; and 402 mg/kg/day and females with doses of 49; 248; 647 mg/kg/day). In this study general toxicity effects were observed, including increased mortality in males of the highest dose group, altered clinical chemistry and hematological parameters, decreased absolute liver and kidney weights, and altered histopathology in these organs. In the juvenile studies no effects on thyroid weight or histopathology were either observed even in the presence of toxicity and at a dose above the MTD.
- *In vitro* mechanistic studies did not show any alteration in any measured parameter, including thyroid receptor (ID 35-38), TSH receptor (ID 64-66), and TRH receptor (ID 63).
- Regarding *in vivo* mechanistic studies, despite a decreased in T4 levels (no dose dependent) in males from the dose of 50 kg/mg/day (ID 32) was observed, this effect was not seen in females (ID 31). In addition, HCD was lower than values observed in control group in this study. No effects on TSH were observed either. Thyroid weight (both sexes) and/or thyroid follicular cell hypertrophy and colloid changes were not observed. The lack of a correlative change in thyroid weight and histopathology, and the fact that T4 decrease was only seen in males, as well as that in longer studies no effects were seen in thyroid, allows to see this alteration as incidental.

Therefore, taking into account the effects observed, it is considered that there is no T-mediated adversity.

2.1.3 Initial analysis of the evidence and identification of relevant scenario for the ED assessment of T-modality

Table 2.10.2.1.3: Selection of relevant scenario

Adversity based on T-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected**
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "**T-mediated**" adversity	X
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no **T-mediated endocrine activity** observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.1.4 Conclusion of the assessment of T-modality

The overall WoE suggests that T-mediated parameters have been sufficiently investigated and T-mediated adversity was not observed across the different studies conducted, at different doses, species, and lengths of treatment. Therefore, the ED criteria are not met for this modality according to a scenario 1a.

2.2. ED assessment for EAS-modalities

2.2.1 Have EAS-mediated parameters been sufficiently investigated?

	Sufficiently investigated
EAS-mediated parameters	No, based on the lack of the following studies: OECD 416 (2001), and OECD 443.

Overall, it is considered that EAS-mediated parameters have not been sufficiently investigated. According to the EFSA/ECHA guidance, the dataset for EAS-mediated adversity for a specific substance is considered sufficient only when studies according to the OECD TG (test guideline) 416 (latest version from 2001) or OECD TG 443 (including the F2 generation) are available (level 5 studies). It is agreed that the dataset can be considered sufficiently investigated also in the case the old version (before 2001) of the OECD TG 416 was applied providing that all relevant parameters, foreseen to be measured according to the new version of OECD TG 416, were measured. However, this is not the case.

According to the EFSA document 'Outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology' (approved in March 2020), the following parameters were considered as a default best scientific practice to be included in the protocol of the study carried out according to the OECD TG 416 i.e. the following parameters should be measured and reported in the study report and then in the DAR/RAR:

- anogenital distance of each F1 and F2 pups,

- presence and number of nipples/areolae in all male F1 and F2 pups,

- histopathological assessment of the mammary gland in P0 and F1 adult males and females,

- sperm parameters measured always by default regardless if they have also been tested in the 90-days.

Among these parameters anogenital distance, sperm parameters, mammary gland of F1 males and females; and presence and number of nipples in all males F1 and F2 pups were not measured.

Except peripubertal assays in female and male rats (US EPA 890.1450 and 890.1500), all studies were conducted according to outdated versions of the guidelines. There are parameters related to endocrine activity that have not been measured, as it is indicated in Table 2.10.2.2.1.

Table 2.10.2.2.1: EAS-mediated parameters not measured

OECD TG 408 and 409 - EAS-mediated parameters not investigated	
- Oestradiol level	- HDL/LDL
- FSH	- Sperm morphology
- LH	- Sperm motility
- Testosterone level	- Sperm numbers
- Epididymis weight	- Vaginal smears
- Oestrus cyclicity	
OECD TG 410 (similar to 407) - EAS-mediated parameters not investigated	
- Cervix histopathology	- Prostate histopathology
- Coagulating gland histopathology	- Seminal vesicles histopathology
- Coagulating gland weight	- Seminal vesicles weight
- Epididymis histopathology	- Testis histopathology
- Epididymis weight	- Uterus histopathology
- Oestrus cyclicity	- Uterus weight
- Mammary gland histopathology (males/females)	- Vagina histopathology
- Ovary weight	- Vaginal smears
OECD TG 414 - EAS-mediated parameters not investigated	
- Anogenital distance measurement	- Gestation length
- Genital abnormalities	- Uterus weight with cervix (gravid uterus)
OECD TG 416 - EAS-mediated parameters not investigated	
- Age at balanopreputial separation	-Seminal vesicles weight
- Age at vaginal opening	- Sperm morphology
- Anogenital distance	- Sperm motility
- Coagulating gland weight	- Sperm numbers
- Epididymis weight	- Uterus weight (with cervix)
OECD TG 453 - EAS-mediated parameters not investigated	
- Epididymis weight	- Uterus weight (with cervix)

It should be also noted that only studies ID 7 (OECD 410), ID 10 (OECD 453), ID 12 (OECD 453), ID 14 (OECD 416), ID 15 (OECD 416), ID 19 (OECD 414), ID 28 (OECD 440), ID 29 and 30 (OECD 441), are considered acceptable and not only as supporting information.

Regarding endocrine activity, the following studies were performed, according to the EFSA/ECHA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009:

E modality: ToxCast Information as well as the Uterotrophic assay (procedure to test for antioestrogenicity was not performed) (OECD 440).

A modality: Hershberger bioassay in rats (OECD 441).

S modality: H295R steroidogenesis assay OECD 456 and the aromatase assay (human recombinant) OPPTS 890.1200.

2.2.2 Lines of evidence for adverse effects and endocrine activity related to EAS-modalities

Table 2.10.2.2.2: Lines of evidence for adverse effects and endocrine activity related to EAS-modality for humans

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
47	In vitro mechanistic	11-Deoxycorticosterone (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect		Some evidence of endocrine activity is observed, including ToxCast estrogen model, ER and AR binding assays and aromatase ans steroidogenesis assays, which gave positive or equivocal results	Overall evidence of EAS mediated activity from *in vitro* studies. *In vivo* studies also indicate alterations, as observed	EAS
48	In vitro mechanistic	11-Deoxycorticosterone (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				
51	In vitro mechanistic	11-Deoxycorticosterone (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	54,72	µM	Decrease	AC50> cytotox limit			
52	In vitro mechanistic	11-Deoxycorticosterone (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				
39	In vitro mechanistic	11-Deoxycortisol (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	45,14	µM	Decrease	AC50> cytotox limit			
40	In vitro mechanistic	11-Deoxycortisol (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				
41	In vitro mechanistic	17-alpha-hydroxypregnelone (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				
42	In vitro mechanistic	17-alpha-hydroxypregnelone (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
43	In vitro mechanistic	17-alpha-hydroxyprogesterone (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	46,36	µM	Decrease	AC50> cytotox limit			
44	In vitro mechanistic	17-alpha-hydroxyprogesterone (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				
22	In vitro mechanistic	Androgen receptor				0		No effect	no agonist 0=no activity; 1=methyltrienolone			
22	In vitro mechanistic	Androgen receptor				0		No effect	no antagonist 0=no activity; 1=hydroxyflutamide			
25	In vitro mechanistic	Androgen receptor	rat prostate cytosol		Uptake from the medium (in vitro)	0,0001	M	Change	OPP was positive for AR binding, RBA = 0.0005-0.0006% of methyltrienolone			
45	In vitro mechanistic	Androstenedione (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				
46	In vitro mechanistic	Androstenedione (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				
49	In vitro mechanistic	Cortisol (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				
50	In vitro mechanistic	Cortisol (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
61	In vitro mechanistic	CYP19	human CYP19A1	0.5 h	Uptake from the medium (in vitro)	4.4	µM	Increase	borderline active			
62	In vitro mechanistic	CYP19	human breast cancer cell line	24 h	Uptake from the medium (in vitro)	50.25	µM	Decrease	AC50 > cytotox limit			
26	In vitro mechanistic	CYP19	human recombinant aromatase	15 min	Uptake from the medium (in vitro)	0.0001	M	Decrease	OPP was positive for aromatase inhibition			
53	In vitro mechanistic	Estradiol level (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				
54	In vitro mechanistic	Estradiol level (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				
27	In vitro mechanistic	Estradiol level (in vitro)	human adrenocortical carcinoma cell line	48 h	Uptake from the medium (in vitro)	1E-05	M	Increase	2.6-fold			
21	In vitro mechanistic	Estrogen receptor				0.0054		Change	inconclusive agonist 0=no activity; 1=17ß-estradiol			
21	In vitro mechanistic	Estrogen receptor				0		No effect	no antagonist 0=no activity; 0.973=Raloxifene			
23	In vitro mechanistic	Estrogen receptor	rat uterine cytosol		Uptake from the medium (in vitro)	0.0004	M	Change	OPP was equivocal for ER binding			
24	In vitro mechanistic	Estrogen receptor	human ER expressed in yeast	84 h	Uptake from the medium (in vitro)	ca. 7E-05	M	Increase	OPP produced a very weak hER activation in the upper µM range			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
55	In vitro mechanistic	Estrone (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				
56	In vitro mechanistic	Estrone (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				
57	In vitro mechanistic	Progesterone (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	41,28	µM	Decrease	AC50 at cytotox limit			
58	In vitro mechanistic	Progesterone (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				
59	In vitro mechanistic	Testosterone level (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				
60	In vitro mechanistic	Testosterone level (in vitro)	human adrenal gland cell line	48 h	Uptake from the medium (in vitro)	>100	µM	No effect				
27	In vitro mechanistic	Testosterone level (in vitro)	human adrenocortical carcinoma cell line	48 h	Uptake from the medium (in vitro)	>1.00E-04	M	No effect	below 1.5x threshold			
29	In vivo mechanistic	Adrenals weight (Hershberger)	rat	10 d	Oral	>1000	mg/kg bw/day	No effect		Signs of alterations in *in vivo* mechanistic studies, mainly in males, showing decreases of accessory sex		
30	In vivo mechanistic	Adrenals weight (Hershberger)	rat	10 d	Oral	>1000	mg/kg bw/day	No effect	17% decrease (no statistically significant)			
29	In vivo mechanistic	Cowpers glands weight (Hershberger)	rat	10 d	Oral	>1000	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
30	In vivo mechanistic	Cowpers glands weight (Hershberger)	rat	10 d	Oral	>1000	mg/kg bw/day	No effect	90% of controls (no statistically significant)	organs and tissues (only statistically significant for ventral prostate).		
29	In vivo mechanistic	Glans penis weight (Hershberger)	rat	10 d	Oral	>1000	mg/kg bw/day	No effect				
30	In vivo mechanistic	Glans penis weight (Hershberger)	rat	10 d	Oral	>1000	mg/kg bw/day	No effect	89% of controls (no statistically significant)			
29	In vivo mechanistic	LABC weight (Hershberger)	rat	10 d	Oral	>1000	mg/kg bw/day	No effect				
30	In vivo mechanistic	LABC weight (Hershberger)	rat	10 d	Oral	>1000	mg/kg bw/day	No effect	96% of controls (no statistically significant)			
29	In vivo mechanistic	Prostate weight (Hershberger)	rat	10 d	Oral	>1000	mg/kg bw/day	No effect				
30	In vivo mechanistic	Prostate weight (Hershberger)	rat	10 d	Oral	1000	mg/kg bw/day	Decrease	72% of controls (the other target tissues displayed some degree of not statistically significant reduced growth)			
29	In vivo mechanistic	Seminal vesicles weight (Hershberger)	rat	10 d	Oral	>1000	mg/kg bw/day	No effect				
30	In vivo mechanistic	Seminal vesicles weight (Hershberger)	rat	10 d	Oral	>1000	mg/kg bw/day	No effect	88% of controls (no statistically significant)			
32	In vivo mechanistic	Testosterone level	rat	PND 23-53	Oral	>900	mg/kg bw/day	No effect				
28e	In vivo mechanistic	Uterus weight (UT assay)	rat	PND 19-22	Oral	1000	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
28d	In vivo mechanistic	Vaginal opening (UT assay)	rat	PND 19-22	Oral	1000	mg/kg bw/day	No effect				
32a	EATS-mediated	Age at balanopreputial separation	rat	PND 23-53	Oral	900	mg/kg bw/day	Increase	Statistically significant delay of 2.1 days. No significant when adjusted for BW on PND23 (It should have been adjusted for PND21)	Delay in males of BPS in pubescent rat	Some evidence of effects in rat, mice, and rabbit. Some of them at doses higher than the MTD. There is a lack of unequivocal EAS adverse effects, however, it is neither possible to discard a EAS pathway.	
31	EATS-mediated	Age at first estrus	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect		No alterations in pubescent females rat in ages at first oestrus and vaginal opening.		
31	EATS-mediated	Age at Vaginal opening	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
10	EATS-mediated	Cervix histopathology	rat	2 yr	Oral	>10'000	ppm	No effect		No alterations in cervix histopathology.		
12	EATS-mediated	Cervix histopathology	mouse	2 yr	Oral	>1000	mg/kg bw/day	No effect				
13	EATS-mediated	Cervix histopathology	mouse	102 wk	Dermal	55.5	other	Increase	1 fibroma vs 0 in the control group (ns)			
14	EATS-mediated	Cervix histopathology	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect				
15	EATS-mediated	Cervix histopathology	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
31	EATS-mediated	Cervix histopathology	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
12	EATS-mediated	Coagulating gland histopathology	mouse	2 yr	Oral	>1000	mg/kg bw/day	No effect		No effects in coagulating gland histopathology.		
15	EATS-mediated	Coagulating gland histopathology	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
5	EATS-mediated	Epididymis histopathology	dog	1 yr	Oral	>300	mg/kg bw/day	No effect		Some alterations were observed at doses above the MTD in epididymis in pubescent rats.		
10	EATS-mediated	Epididymis histopathology	rat	2 yr	Oral	>8000	ppm	No effect				
12	EATS-mediated	Epididymis histopathology	mouse	2 yr	Oral	>1000	mg/kg bw/day	No effect				
13	EATS-mediated	Epididymis histopathology	mouse	102 wk	Dermal	55.5	other	No effect				
14	EATS-mediated	Epididymis histopathology	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect				
15	EATS-mediated	Epididymis histopathology	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
32	EATS-mediated	Epididymis histopathology	rat	PND 23-53	Oral	>900	mg/kg bw/day	No effect	Immature and decreased spermatic elements were noted in the right epididymis of some control and treated animals.			
32	EATS-mediated	Epididymis weight	rat	PND 23-53	Oral	900	mg/kg bw/day	Decrease	Decrease adjusted weight of right and left epididymides (4% and 6%, respectively) at the highest dose.			
14	EATS-mediated	Estrus cyclicity	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect		Oestrus cyclicity was altered in pubertal assay at a dose above MTD. In 2 generation studies some deviations were observed,		
15	EATS-mediated	Estrus cyclicity	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
31	EATS-mediated	Estrus cyclicity	rat	PND 22-42	Oral	900	mg/kg bw/day	Change	Regular cycling (900 mg/kg/day vs control group, respectively): 28.6% vs. 86.7%			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									(4 vs 13); % cycling: 64.3% vs 93.3% (9 vs 14). Mean cycle days (control vs 900 mg/kg/day) 4.7 vs 5.7 (not statistically different).	therefore not fully reliable outcomes can be extracted.		
32	EATS-mediated	LABC weight	rat	PND 23-53	Oral	900	mg/kg bw/day	Decrease	Decrease adjusted and unadjusted weight (16% and 18%, respectively) at the highest dose.	Decrease in LABC weight.		
5	EATS-mediated	Mammary gland histopathology (female)	dog	1 yr	Oral	>300	mg/kg bw/day	No effect		No effects in mammary gland histopathology were observed in males or females.		
10	EATS-mediated	Mammary gland histopathology (female)	rat	2 yr	Oral	>10'000	ppm	No effect				
12	EATS-mediated	Mammary gland histopathology (female)	mouse	2 yr	Oral	1000	mg/kg bw/day	Increase	0 vs 1 at the highest dose			
13	EATS-mediated	Mammary gland histopathology (female)	mouse	102 wk	Dermal	>55.5	other	No effect				
31	EATS-mediated	Mammary gland histopathology (female)	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
5	EATS-mediated	Mammary gland histopathology (male)	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
10	EATS-mediated	Mammary gland histopathology (male)	rat	2 yr	Oral	>8000	ppm	No effect				
12	EATS-mediated	Mammary gland histopathology	mouse	2 yr	Oral	>1000	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		(male)										
4	EATS-mediated	Ovary histopathology	rat	13 wk	Oral	>25'000	ppm	No effect		Ovary did not show consistent alterations.		
5	EATS-mediated	Ovary histopathology	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
6	EATS-mediated	Ovary histopathology	dog	1 yr	Oral	>500	mg/kg bw/day	No effect				
8	EATS-mediated	Ovary histopathology	mouse	4 wk	Dermal	>55.5	mg/0.1 mL	No effect				
10	EATS-mediated	Ovary histopathology	rat	2 yr	Oral	>10'000	ppm	No effect				
12	EATS-mediated	Ovary histopathology	mouse	2 yr	Oral	1000	mg/kg bw/day	Increase				
13	EATS-mediated	Ovary histopathology	mouse	102 wk	Dermal	55.5	other	Increase	Follicular cyst 17 vs 32; luteoma 1 vs 3			
14	EATS-mediated	Ovary histopathology	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect				
15	EATS-mediated	Ovary histopathology	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
31	EATS-mediated	Ovary histopathology	rat	PND 22-42	Oral	900	mg/kg bw/day	Induction	One rat given 900 mg/kg/day had juvenile appearance of the ovary (B.W of this animal was less than 17% of the mean group)			
4	EATS-mediated	Ovary weight	rat	13 wk	Oral	>25'000	ppm	No effect				
5	EATS-mediated	Ovary weight	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
10	EATS-mediated	Ovary weight	rat	2 yr	Oral	>10'000	ppm	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
14	EATS-mediated	Ovary weight	rat	15/10 (P/F1) wk	Oral	457	mg/kg bw/day	Increase	+33% increase in relative ovary weight in P females, but control ovary weight was unusually low in F0 females			
15	EATS-mediated	Ovary weight	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
31	EATS-mediated	Ovary weight	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
5	EATS-mediated	Oviduct histopathology	dog	1 yr	Oral	>300	mg/kg bw/day	No effect		Oviduct histopathology was altered in one study in mouse.		
12	EATS-mediated	Oviduct histopathology	mouse	2 yr	Oral	1000	mg/kg bw/day	Increase	cyst 1 vs 3 at the highest dose (not analyzed statistically).			
13	EATS-mediated	Oviduct histopathology	mouse	102 wk	Dermal	>55.5	other	No effect				
31	EATS-mediated	Oviduct histopathology	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
4	EATS-mediated	Prostate histopathology (with seminal vesicles and coagulating glands)	rat	13 wk	Oral	>25'000	ppm	No effect		Prostate histopathology was not altered. Prostate weight was decreased in peripubertal male assay at a dose above MTD.		
5	EATS-mediated	Prostate histopathology (with seminal vesicles and coagulating glands)	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
10	EATS-mediated	Prostate histopathology (with seminal vesicles and	rat	2 yr	Oral	>8000	ppm	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		coagulating glands)										
12	EATS-mediated	Prostate histopathology (with seminal vesicles and coagulating glands)	mouse	2 yr	Oral	>1000	mg/kg bw/day	No effect				
13	EATS-mediated	Prostate histopathology (with seminal vesicles and coagulating glands)	mouse	102 wk	Dermal	>55.5	other	No effect				
14	EATS-mediated	Prostate histopathology (with seminal vesicles and coagulating glands)	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect				
15	EATS-mediated	Prostate histopathology (with seminal vesicles and coagulating glands)	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
4	EATS-mediated	Prostate weight	rat	13 wk	Oral	>25'000	ppm	No effect				
5	EATS-mediated	Prostate weight	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
32	EATS-mediated	Prostate weight	rat	PND 23-53	Oral	>900	mg/kg bw/day	Decrease	Both adjusted and unadjusted weight of ventral prostate decreased (-17%; -20%, respectively)			
32	EATS-mediated	Prostate weight	rat	PND 23-53	Oral	>900	mg/kg bw/day	No effect	Dorsolateral prostate weight			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									decreased, but not in a statistically significantly way at the highest dose (11% and 15%, adjusted and unadjusted weight, respectively).			
10	EATS-mediated	Seminal vesicles histopathology	rat	2 yr	Oral	>8000	ppm	No effect		No consistent effects in seminal vesicles histopathology. Effects in weight at a dose above MTD.		
12	EATS-mediated	Seminal vesicles histopathology	mouse	2 yr	Oral	>1000	mg/kg bw/day	No effect				
13	EATS-mediated	Seminal vesicles histopathology	mouse	102 wk	Dermal	>55.5	other	No effect				
14	EATS-mediated	Seminal vesicles histopathology	rat	15/10 (P/F1) Wk	Oral	457	mg/kg bw/day	No effect	No significant increase in secretion, hypercellular 4/35 vs 7/35			
15	EATS-mediated	Seminal vesicles histopathology	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
32	EATS-mediated	Seminal vesicles weight	rat	PND 23-53	Oral	900	mg/kg bw/day	Decrease	Adjusted weight of coagulating gland, without fluid, was decreased 14%. Both adjusted and unadjusted weight of coagulating gland, with fluid, were decreased (19% and 23%, respectively).			
3	EATS-mediated	Testis histopathology	rat	3 mo	Oral	>20'000	ppm	No effect		Testis histopathology and weight alterations		
4	EATS-mediated	Testis histopathology	rat	13 wk	Oral	>25'000	ppm	No effect				

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5	EATS-mediated	Testis histopathology	dog	1 yr	Oral	>300	mg/kg bw/day	No effect		were observed in some studies longer than three months in rat and mouse. Except in studies ID 9 and 15, alterations in weight were also observed. In study ID 14, alterations in weight were transitory		
6	EATS-mediated	Testis histopathology	dog	1 yr	Oral	>500	mg/kg bw/day	No effect				
9	EATS-mediated	Testis histopathology	rat	2 yr	Oral	20'000	ppm	No effect	Not specified (at the highest dose)			
10	EATS-mediated	Testis histopathology	rat	2 yr	Oral	>8000	ppm	No effect				
11	EATS-mediated	Testis histopathology	rat	91 wk	Oral	1140	mg/kg/day	Increase	At 1140 mg/kg bw/day, interstitial cell tumours of the testes were the tumours most frequently observed other than urinary bladder (no more specification).			
12	EATS-mediated	Testis histopathology	mouse	2 yr	Oral	1000	mg/kg bw/day	Increase	Leydig cell tumour 1 in the control group vs 2 at the highest dose (not statistically significant)			
13	EATS-mediated	Testis histopathology	mouse	102 wk	Dermal	55.5	other	Increase	1 interstitial cell tumour; 1 adenoma (ns)			
14	EATS-mediated	Testis histopathology	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect				
15	EATS-mediated	Testis histopathology	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
32	EATS-mediated	Testis histopathology	rat	PND 23-53	Oral	>900	mg/kg bw/day	No effect	Testis histopathology was performed on right testis, which			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									did not show any alteration in weight, contraty to left testis.			
4	EATS-mediated	Testis weight	rat	13 wk	Oral	25'000	ppm	Increase	+20% increase in testis relative weight at the highest dose. No statistical change in absolute weight.			
5	EATS-mediated	Testis weight	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
7	EATS-mediated	Testis weight	rat	3 wk	Dermal	>1000	mg/kg bw/day	No effect				
9	EATS-mediated	Testis weight	rat	2 yr	Oral	20'000	ppm	Increase	46% increase in relative testis weight at the highest dose.			
10	EATS-mediated	Testis weight	rat	2 yr	Oral	8000	ppm	Increase	Increase of 34% of testes absolute weight in the 402 mg/kg/day group (8000 ppm) at the end of the treatment. Increase of 46% in the relative weight.			
12	EATS-mediated	Testis weight	mouse	2 yr	Oral	1000	mg/kg bw/day	Increase	Increase in 14% of testes relative weight at the dose of 1000 mg/kg/day. No change in absolute weight			
14	EATS-mediated	Testis weight	rat	15/10 (P/F1)	Oral	457	mg/kg bw/day	Increase	Increase relative testis weight			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
				wk					(49%) at the dose of 457 mg/kg/day in F1 males. No change in absolute weight.			
15	EATS-mediated	Testis weight	rat	10 wk	Oral	458	mg/kg bw/day	Increase	Elevated relative testes weights in the high-dose group F1 males (12% above the control group). Not considered to be compound-related since the absolute testes weights were similar to controls. This relative testis weight increase was associated with a concurrent decrease in terminal body weight for the high-dose group F1 males.			
32	EATS-mediated	Testis weight	rat	PND 23-53	Oral	900	mg/kg bw/day	Decrease	Decrease adjusted and unadjusted weight of left testis (7% and 9%, respectively) at the highest dose. Right testis did not vary.			
4	EATS-mediated	Uterus histopathology (with cervix)	rat	13 wk	Oral	>25'000	ppm	No effect		No alterations in uterus weight or		

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
5	EATS-mediated	Uterus histopathology (with cervix)	dog	1 yr	Oral	>300	mg/kg bw/day	No effect		histopathology were observed at doses below MTD.		
6	EATS-mediated	Uterus histopathology (with cervix)	dog	1 yr	Oral	>500	mg/kg bw/day	No effect				
10	EATS-mediated	Uterus histopathology (with cervix)	rat	2 yr	Oral	>10'000	ppm	No effect				
12	EATS-mediated	Uterus histopathology (with cervix)	mouse	2 yr	Oral	>1000	mg/kg bw/day	No effect				
13	EATS-mediated	Uterus histopathology (with cervix)	mouse	102 wk	Dermal	>55.5	other	No effect				
14	EATS-mediated	Uterus histopathology (with cervix)	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect				
15	EATS-mediated	Uterus histopathology (with cervix)	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
31	EATS-mediated	Uterus histopathology (with cervix)	rat	PND 22-42	Oral	900	mg/kg bw/day	Induction	Two rats given 900 mg/kg/day had very slight decreased size of the uterus (B.W of these animals were less than 17-19% of the mean group)			
4	EATS-mediated	Uterus weight (with cervix)	rat	13 wk	Oral	>25'000	ppm	No effect				
5	EATS-mediated	Uterus weight (with cervix)	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
5	EATS-mediated	Vagina histopathology	dog	1 yr	Oral	>300	mg/kg bw/day	No effect		Alterations in vagina histopathology		
10	EATS-	Vagina	rat	2 yr	Oral	>10'000	ppm	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	mediated	histopathology								were only observed in mouse.		
12	EATS-mediated	Vagina histopathology	mouse	2 yr	Oral	500	mg/kg bw/day	Increase	Incidence of Mass/Nodule: 1 at the dose of 500 mg/kg/day and 2 at the dose of 1000 mg/kg/day (0 in the control).			
14	EATS-mediated	Vagina histopathology	rat	15/10 (P/F1) wk	Oral	>457	mg/kg bw/day	No effect				
15	EATS-mediated	Vagina histopathology	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
31	EATS-mediated	Vagina histopathology	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
3	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	rat	3 mo	Oral	>20'000	ppm	No effect		No consistent effects on adrenal histopathology. Adrenal weight alterations were not correlated to histological changes except in the dermal 2-year study in mice. Increases in adrenal weight seem to occur in males and decreases in females.		
4	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	rat	13 wk	Oral	>25'000	ppm	No effect				
5	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
6	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	dog	1 yr	Oral	>500	mg/kg bw/day	No effect				
9	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	rat	2 yr	Oral	20'000	ppm	No effect	Not specified (at the highest dose)			
10	Sensitive to, but not	Adrenals histopathology	rat	2 yr	Oral	>8000/>10'000	ppm	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	diagnostic of, EATS											
12	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	mouse	2 yr	Oral	>1000	mg/kg bw/day	No effect				
13	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	mouse	102 wk	Dermal	>55.5	other	Increase	Increased incidences of lipoid degeneration in the zona fasciculata of the adrenal gland in 1/49 vehicle control, 4/45 o-phenylphenol, male mice and in 4/50 vehicle control, 24/47 o-phenylphenol female mice.			
31	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
32	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
4	Sensitive to, but not diagnostic of, EATS	Adrenals weight	rat	13 wk	Oral	25'000	ppm	Increase	+22%/+9.8% (m/f) increase in adrenal relative weight at the highest dose.			
5	Sensitive to, but not diagnostic of, EATS	Adrenals weight	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
9	Sensitive to, but not diagnostic of, EATS	Adrenals weight	rat	2 yr	Oral	20'000	ppm	No effect				
10	Sensitive to, but not diagnostic of, EATS	Adrenals weight	rat	2 yr	Oral	10'000	ppm	Decrease	Decrease in females adrenal weight at the dose of 647 mg/kg/day (10000 ppm) of 13.6%. No change in relative weight.			
12a	Sensitive to, but not diagnostic of, EATS	Adrenals weight	mouse	2 yr	Oral	250	mg/kg bw/day	Increase	+16%; 18%; and 50% increase in males in relative adrenal weight. Increase of 33% in adrenal absolute weight at the dose of 1000 mg/kg/day			
31	Sensitive to, but not diagnostic of, EATS	Adrenals weight	rat	PND 22-42	Oral	900	mg/kg bw/day	Decrease	-12.8% adjusted weight. Relative or unadjusted weight did not vary.			
32	Sensitive to, but not diagnostic of, EATS	Adrenals weight	rat	PND 23-53	Oral	250	mg/kg bw/day	Increase	Increase of 16% in absolute weight at 900 mg/kg/day. Increase at the two highest doses in the adjusted weight (for PND23) of 9% and 11%, respectively.			
4	Sensitive to, but not	Brain weight	rat	13 wk	Oral	25'000	ppm	Change	Decrease of 5% in absolute brain	Alterations in brain weight		

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	diagnostic of, EATS								weight and increase of 18% in relative brain weight at the dose of 25'000 ppm in males. 10% increase in relative brain weight in females at the highest dose.	that could be associated to decreases in body weight.		
7	Sensitive to, but not diagnostic of, EATS	Brain weight	rat	3 wk	Dermal	>1000	mg/kg bw/day	No effect				
10	Sensitive to, but not diagnostic of, EATS	Brain weight	rat	2 yr	Oral	10’000	ppm	Increase	Increase in relative brain weight in males and females at the top dose of 402/647 mg/kg/day, respectively, of 7.8% and 18%.			
12	Sensitive to, but not diagnostic of, EATS	Brain weight	mouse	2 yr	Oral	500	mg/kg bw/day		Increases in relative brain weight in males and females of the top doses of 500 and 1000 mg/kg/day of 10% and 15% in males; and 15% and 23% in females, respectively			
14	Sensitive to, but not	Fertility (mammals)	rat	15/10 (P/F1)	Oral	>457	mg/kg bw/day	No effect		Fertility index was decreased		

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	diagnostic of, EATS			wk						in mouse. However, control groups of rat studies showed abnormally low fertility index. Therefore, this fact could be masking low fertilities in treated groups. In addition, some deviation were noted in the determination of fertility.		
15	Sensitive to, but not diagnostic of, EATS	Fertility (mammals)	rat	10 wk	Oral	>458	mg/kg bw/day	Increase	Increased fertility index in one of the two F2 groups (31%). (This was attributed to the abnormally low control value).			
18	Sensitive to, but not diagnostic of, EATS	Fertility (mammals)	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Fertility (mammals)	mouse	GD 7-15	Oral	2100	mg/kg bw/day	Increase	Fertility index: 14/21; 14/21 1450; 5/21; at the doses of 1740; and 2100 mg/kg/day, respectively. In control group 20/21			
14	Sensitive to, but not diagnostic of, EATS	Gestation length	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect		No effects on gestation length were observed.		
15	Sensitive to, but not diagnostic of, EATS	Gestation length	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
16	Sensitive to, but not diagnostic of, EATS	Litter size	rat	GD 6-15	Oral	>700	mg/kg bw/day	No effect		No effects observed in litter size.		
18	Sensitive to, but not diagnostic of, EATS	Litter size	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
19	Sensitive to, but not diagnostic of, EATS	Litter size	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Litter size	mouse	GD 7-15	Oral	>2100	mg/kg bw/day	No effect				
18	Sensitive to, but not diagnostic of, EATS	Litter viability	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	No effect		No effects observed in litter viability		
19	Sensitive to, but not diagnostic of, EATS	Litter viability	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Litter viability	mouse	GD 7-15	Oral	>2100	mg/kg bw/day	No effect	There was no significant difference and no dose dependence in respect of quantity.			
15	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	rat	10 wk	Oral	457	mg/kg bw/day	Decrease	At the dose of 458 mg/kg/day, decrease at day 21, in F1 pups' weights (12% and 10% in both groups), and in F2 at days 14 (5.7% and 4%) and at day 21 (10.6% and 12%).	Decreases in mouse and rat litter/pup weight in prenatal and 2 generation studies. Decreases in maternal body weight gain were observed.		
16	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	rat	GD 6-15	Oral	>700	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
17	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	rat	GD 6-15	Oral	600	mg/kg bw/day	Decrease	6% decrease in males and 8.5% decrease in females at the dose of 600 mg/kg/day. 27% decrease in males and 27% decrease in females at the dose of 1200 mg/kg/day.			
19	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	mouse	GD 7-15	Oral	1450	mg/kg bw/day	Decrease	Body weight of the live foetuses of both sexes was significantly reduced and a retardation of development must be assumed.			
16	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	rat	GD 6-15	Oral	>700	mg/kg bw/day	No effect		Decreased implantations in a developmental study in rat. However, this effect may be disregarded due to methodological deficiencies, as explained in EAS WoE section.		
17	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	rat	GD 6-15	Oral	>1200	mg/kg bw/day	Decrease	At the dose of 1200 mg/kg/day, 8 vs 11.5 in control group, only one litter at 1200 mkd			
18	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
19	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	mouse	GD 7-15	Oral	>2100	mg/kg bw/day	No effect				
14	Sensitive to, but not diagnostic of, EATS	Number of live births	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect		No significant effects on live births were observed		
15	Sensitive to, but not diagnostic of, EATS	Number of live births	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
16	Sensitive to, but not diagnostic of, EATS	Number of live births	rat	GD 6-15	Oral	>700	mg/kg bw/day	No effect				
17	Sensitive to, but not diagnostic of, EATS	Number of live births	rat	GD 6-15	Oral	1200	mg/kg bw/day	Decrease	At the dose of 1200 mg/kg/day, 8 vs 11.5 in control group, only one litter at 1200 mkd			
5	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	dog	1 yr	Oral	>300	mg/kg bw/day	No effect		Pituitary histopathology was not significantly altered. Pituitary weight was decreased at the highest dose in the pubertal rat assays. IN		
10	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	rat	2 yr	Oral	>8000/>10'000	ppm	No effect				
12	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	mouse	2 yr	Oral	>1000	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
13	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	mouse	102 wk	Dermal	>55.5	other	No effect		males the decrease was in absolute and adjusted weight, and in females in the relative weight.		
14	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	rat	15/10 (P/F1) wk	Oral	>457	mg/kg bw/day	No effect				
15	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
31	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
32	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	rat	PND 22-42	Oral	900	mg/kg bw/day	Increase	1 animal presented pale pituitary at the highest dose			
5	Sensitive to, but not diagnostic of, EATS	Pituitary weight	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
31	Sensitive to, but not diagnostic of, EATS	Pituitary weight	rat	PND 22-42	Oral	900	mg/kg bw/day	Decrease	9.8% less relative weight. Adjusted and unadjusted weight did not statistically vary.			
32	Sensitive to, but not diagnostic of, EATS	Pituitary weight	rat	PND 23-53	Oral	900	mg/kg bw/day	Decrease	Decrease in absolute and adjusted weight (PND23) at the highest dose (-15% and -11%, respectively)			
17	Sensitive to, but not	Post implantation loss	rat	GD 6-15	Oral	600	mg/kg bw/day	Increase	At the dose of 600 mg/kg/day	Some increases in		

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	diagnostic of, EATS								25.7% vs. 13.9% in the control group. 38,5% in the 1200 mg/kg/day.	post implantation loss were observed. As explained WoE section of EAS modalities, deviations in the test methods may be minimising their incidence.		
18	Sensitive to, but not diagnostic of, EATS	Post implantation loss	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	No effect				
19	Sensitive to, but not diagnostic of, EATS	Post implantation loss	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Post implantation loss	mouse	GD 7-15	Oral	>2100	mg/kg bw/day	No effect				
16	Sensitive to, but not diagnostic of, EATS	Pre implantation loss	rat	GD 6-15	Oral	>700	mg/kg bw/day	No effect				
18	Sensitive to, but not diagnostic of, EATS	Pre implantation loss	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	No effect				
19	Sensitive to, but not diagnostic of, EATS	Pre implantation loss	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Pre implantation loss	mouse	GD 7-15	Oral	1450	mg/kg bw/day	Increase				
16	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	rat	GD 6-15	Oral	700	mg/kg bw/day	Change	delayed ossification of skull, pinpoint holes in the occipital or	Increased incidence of anomalies was noted in rodents.		

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									interparietal plates in the skull, and skull bone island (outside HCD) delayed ossification of sternebrae (inside HCD)			
17	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	rat	GD 6-15	Oral	300	mg/kg bw/day	Increase	Of the foetuses from 300 or 600 mg/kg group, only 1 or 2 showed concurrent occurrence of anomalies such as cranial or sacral meningocele and diaphragmatic hernia. However, the anomalies were too low in their incidences to be analysed by this study whether they were caused by OPP or not. A decrease in the maternal food-intake during the period of the treatment might contribute to the occurrence of the anomalies. Foetuses survived their maternal treatment with			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									1200 mg/kg of OPP were free from anomalies.			
19	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	mouse	GD 7-15	Oral	1450	mg/kg bw/day	Change	There was a tendency, though not a significant one, for the number of cervical ribs to increase in a manner dependent on the dose.			
14	Sensitive to, but not diagnostic of, EATS	Pup survival index	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect		No effects on pup survival index		
15	Sensitive to, but not diagnostic of, EATS	Pup survival index	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
14	Sensitive to, but not diagnostic of, EATS	Sex ratio	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect		No alterations on sex ratio were observed.		
15	Sensitive to, but not diagnostic of, EATS	Sex ratio	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
16	Sensitive to, but not diagnostic of, EATS	Sex ratio	rat	GD 6-15	Oral	>700	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
19	Sensitive to, but not diagnostic of, EATS	Sex ratio	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Sensitive to, but not diagnostic of, EATS	Sex ratio	mouse	GD 7-15	Oral	>2100	mg/kg bw/day	No effect				
14	Sensitive to, but not diagnostic of, EATS	Time to mating	rat	15/10 (P/F1) Wk	Oral	>457	mg/kg bw/day	No effect				
15	Sensitive to, but not diagnostic of, EATS	Time to mating	rat	10 wk	Oral	>458	mg/kg bw/day	No effect				
4	Target organ toxicity	Kidney histopathology	rat	13 wk	Oral	25'000	ppm	Change	Inflammation in kidney at the highest dose	Decreases in absolute kidney weight, mainly in long term studies. Increases in histopathological alterations mainly at high doses.	Overall evidence of effects in kidney and liver.	
6	Target organ toxicity	Kidney histopathology	dog	1 yr	Oral	>500	mg/kg bw/day	No effect				
7	Target organ toxicity	Kidney histopathology	rat	3 wk	Dermal	>1000	mg/kg bw/day	No effect				
9	Target organ toxicity	Kidney histopathology	rat	2 yr	Oral	20'000	ppm	Increase	Extensive renal damage, characterised by tubular dilatation with varying degrees of acute and chronic inflammation			
10	Target organ toxicity	Kidney histopathology	rat	2 yr	Oral	10000	ppm	Induction	7 females of the dose of 647 mg/kg/day (10'000 ppm) vs 0 in control group			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									presented pitted zones and 8 vs 1 presented abnormal texture. Increased incidence of renal infarct (29 vs 3) in females; hyperolasia (30 vs 3) in females; cyst in males (17 vs 4) and females (37 vs 14); acute inflammation (M: 7, 11, 3, 5; F: 2, 0, 0, 11 *) and in the incidence of mineralization within the tubules of the renal papilla was noted (F:0,0,2,12*) in 10,000 ppm females.			
11	Target organ toxicity	Kidney histopathology	rat	91 week	Oral	12’500 ppm (531 mg/kg/day)	ppm	Induction	Moderate to severe nephritic lesions appeared in 3/24 (13%) of the 1.25% group and 23/23 (100%) of the 2.5% group. The incidence of this lesion was significantly higher in the 2.5% group than in the controls.			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									Among these lesions, moderate to severe pyelonephritis with papillary destruction were found in 1/3 (33%) of the 1.25% and 15/23 (65%) of the 2.5% groups, and the other lesion was interstitial nephritis.			
12	Target organ toxicity	Kidney histopathology	mouse	2 yr	Oral	250	mg/kg bw/day	Increase	A dose-related decrease in the incidence of microvacuolation in the kidney tubules of male mice was observed at all dose levels.			
14	Target organ toxicity	Kidney histopathology	rat	15/10 (P/F1) wk	Oral	457	mg/kg bw/day	Decrease	In P males at the highest dose, increase in calculus (13 vs 3) and hemorrhage (6 vs 0)			
15	Target organ toxicity	Kidney histopathology	rat	10 wk	Oral	458	mg/kg bw/day	Increase	P and F1 males did appear to have a greater number of animals with numerous background lesions, multiple lesions with			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									severity grades of slight to marked, and/or lesions such as chronic active inflammation and debris in the renal pelvis that were noted only in the high-dose level males (no statistically significant)			
18	Target organ toxicity	Kidney histopathology	rabbit	GD 7-19	Oral	500	mg/kg bw/day	Change	dose dependent alterations			
19	Target organ toxicity	Kidney histopathology	rabbit	GD 7-19	Oral	250	mg/kg bw/day	Change	Treatment-related effects on the kidneys were observed in 10 of 24 (42%) rabbits at 250 mg/kg/day. The kidneys had tubular degeneration, focal to multifocal in distribution, slight to moderate in degree, accompanied by inflammation that was focal to multifocal in distribution, and slight in degree.			
31	Target organ	Kidney histopathology	rat	PND 22-42	Oral	900	mg/kg bw/day	Induction	very slight or slight focal or			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	toxicity								multifocal dilation of the renal tubule (2 vs 11 in the control group and 900 mg/kg/day, respectively), sometimes accompanied by degeneration and necrosis (0 vs 2); slight hyperplasia of the epithelium lining the papilla and very slight hypertrophy of the epithelial cells (0 vs 1 in the control and 900 mg/kg/day, respectively) lining the collecting duc.			
32	Target organ toxicity	Kidney histopathology	rat	PND 23-53	Oral	900	mg/kg bw/day	Increase	Control vs 900 mg/kg/day group effects: Dilatation, tubule, focal/multifocal – Very slight or Slight (4 vs 12, respectively); Hypertrophy, collecting duct, epithelium, focal/multifocal – Very slight (0 vs 5, respectively); hyperplasia,			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									epithelium, papilla, unilateral or bilateral, multifocal –Very slight (0 vs 2)			
1	Target organ toxicity	Kidney weight	rabbit	13 d	Oral	100	mg/kg bw/day	No effect				
4	Target organ toxicity	Kidney weight	rat	13 wk	Oral	6250	ppm	Increase	Increases of 4.3%; 5.7%; and 25% in the kidney relative weight in males at the doses of 6250, 12'500, 25'000 ppm, respectively. No changes in absolute weight. In females, increase of 15% in the relative kidney weight at the highest dose.			
5	Target organ toxicity	Kidney weight	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
6	Target organ toxicity	Kidney weight	dog	1 yr	Oral	500	mg/kg bw/day	Increase	Slight increase in kidney weight at the top dose of 500 mg/kg/day (not specified)			
7	Target organ toxicity	Kidney weight	rat	3 wk	Dermal	>1000	mg/kg bw/day	No effect				
10	Target organ toxicity	Kidney weight	rat	2 yr	Oral	4000	ppm	Decrease	Decreased kidney weight in females at the doses of 8% and 11% at the			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									doses of 248 and 647 mg/kg/day, respectively (4000 and 10000 ppm). No change in relative weight.			
12	Target organ toxicity	Kidney weight	mouse	2 yr	Oral	500	mg/kg bw/day		Decrease in males in absolute kidney weight of 7% and 14% at the doses of 500 and 1000 mg/kg/day, respectively. And increases in relative kidney weight in females 17% and 20% at the highest doses.			
14	Target organ toxicity	Kidney weight	rat	15/10 (P/F1) wk	Oral	457	mg/kg bw/day	Increase	At the highest dose of 457 mg/kg/day, increase in P and F1 relative kidney weights in males (8% and 11%, respectively). Decrease in absolute kidney weights in P females (9.4%).			
18	Target organ toxicity	Kidney weight	rabbit	GD 7-19	Oral	500	mg/kg bw/day	Increase	Increased relative weight (34%) at the dose of 500 mg/kg/day (the highest dose at which this parameter was measured).			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
19	Target organ toxicity	Kidney weight	rabbit	GD 7-19	Oral	>250	mg/kg bw/day	No effect				
31	Target organ toxicity	Kidney weight	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect				
32	Target organ toxicity	Kidney weight	rat	PND 23-53	Oral	>900	mg/kg bw/day	No effect				
3	Target organ toxicity	Liver histopathology	rat	3 mo	Oral	>20'000	ppm	No effect		Alterations in liver weight in rodents in studies longer than 90 days except in one. Dams seem to present a light tendency to have a decrease in liver weight is observed in the developmental studies and in F1 animals of one two generation study. Histological findings were observed in two long term studies at the highest doses and in the only prenatal developmental		
4	Target organ toxicity	Liver histopathology	rat	13 wk	Oral	25’000	ppm	No effect				
5	Target organ toxicity	Liver histopathology	dog	1 yr	Oral	300	mg/kg/day	No effect				
6	Target organ toxicity	Liver histopathology	dog	1 yr	Oral	>500	mg/kg bw/day	No effect				
7	Target organ toxicity	Liver histopathology	rat	3 wk	Dermal	>1000	mg/kg bw/day	No effect	Not specified (at the highest dose)			
8	Target organ toxicity	Liver histopathology	mouse	4 wk	Dermal	55.5 mg/0.1 mL	mg/mL	No effect				
9	Target organ toxicity	Liver histopathology	rat	2 yr	Oral	20'000	ppm	Increase	Not specified (at the highest dose)			
10	Target organ toxicity	Liver histopathology	rat	2 yr	Oral	>10000 (402 mg/kg/day males/ 647 mg/kg/day for females)	ppm	No effect				
12	Target organ	Liver histopathology	mouse	2 yr	Oral	500	mg/kg bw/day	Increase	Gross necropsy observations in			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	toxicity								the middle and high dose males, suggested a slight increase in the number of mice with a liver mass/nodule. A dose-related increased in the incidence of "accentuated lobular pattern" was observed at all dose levels in both sexes. Incidence of male mice with hepatocellular adenoma was statistically significantly increased in the middle and high dose groups.	study that it was measured.		
14	Target organ toxicity	Liver histopathology	rat	15/10 wk (P/F1) wk	Oral	>457	mg/kg bw/day	No effect				
15	Target organ toxicity	Liver histopathology	rat	10 wk	Oral	458	mg/kg bw/day	Increase	At the dose of 458 mg/kg/day, 2 F1 males showed malignant lymphoma and 1 male showed necrosis (not statistically significant)			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
18	Target organ toxicity	Liver histopathology	rabbit	GD 7-19	Oral	250	mg/kg bw/day	Increase	1; 2; 5 animals presented autolysis vs 0 in the control group.			
1c	Target organ toxicity	Liver weight	rabbit	13 d	Oral	>1000	mg/kg bw/day	No effect				
3	Target organ toxicity	Liver weight	rat	3 mo	Oral	10’000	ppm	Increase	Increases in liver weight at the doses of 10'000 and 20'000 ppm			
4	Target organ toxicity	Liver weight	rat	13 wk	Oral	3130	ppm	Increase	Increases in males of 7%; 7.3%; 11%; 20% in relative liver weight at the doses of 3130, 6250, 12'500, 25'000, respectively. No changes in absolute liver weights. In females relative increases of 13%; and 33% at the two highest doses, respectively. Increase of 15% at the highest dose in absolute liver weight in females.			
5	Target organ toxicity	Liver weight	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
7	Target organ toxicity	Liver weight	rat	3 wk	Dermal	>1000	mg/kg bw/day	No effect				
9	Target organ toxicity	Liver weight	rat	2 yr	Oral	>20’000	ppm	No effect				
10	Target organ toxicity	Liver weight	rat	2 yr	Oral	4000 ppm (248 mg/kg/day)	ppm	Decrease	Decreased liver weight in females at the doses of 9.5% and 12.5% at the doses of 248 and 647 mg/kg/day, respectively (4000 and 10000 ppm). No change in relative weight.			
12	Target organ toxicity	Liver weight	mouse	2 yr	Oral	500	mg/kg bw/day	Increase	Increase in females in absolute liver weight of 36% and 23% at the doses of 500 and 1000 mg/kg/day, respectively. Increase of liver relative weight 16%; 56%; and 46% at 250, 500 and 1000 mg/kg/day, respectively.			
14	Target organ toxicity	Liver weight	rat	15/10 (P/F1) wk	Oral	457	mg/kg bw/day	Decrease	Decreased absolute liver weight (13.4%) in F1 females at the dose of 457 mg/kg/day			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
16a	Target organ toxicity	Liver weight	rat	GD 6-15	Oral	700	mg/kg bw/day	Decrease	At the dose of 700 mg/kg/day, absolute liver weight decreased 17%. Relative weight did not change.			
18	Target organ toxicity	Liver weight	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	No effect				
19	Target organ toxicity	Liver weight	rabbit	GD 7-19	Oral	>250	mg/kg bw/day	No effect				
31	Target organ toxicity	Liver weight	rat	PND 22-42	Oral	900	mg/kg bw/day	Increase	+9.3% relative to BW. Adjusted and unadjusted weight did not vary.			
32	Target organ toxicity	Liver weight	rat	PND 23-53	Oral	250	mg/kg bw/day	Increase	+8% and +21% in relative liver weight at the doses of 250 and 900 mg/kg/day; increase at the highest dose of adjusted (for PND 23) weight of 10%. No difference in unadjusted weight			
1a	Systemic toxicity	Body weight	rabbit	13 d	Oral	100	mg/kg bw/day	Decrease	Decreased BW (24%) at the highest dose of 1000 mg/kg/day.	Signs of systemic toxicity occurred at high doses, which included mainly clinical signs, effects	Overall evidence of systemic toxicity.	
2	Systemic toxicity	Body weight	dog	4 wk	Oral	300	mg/kg bw/day	Decrease	decreased BW gain in females at the dose of 300 mg/kg/day			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
3	Systemic toxicity	Body weight	rat	3 mo	Oral	20'000	ppm	Increase	Slight decrease in gain weight at the highest dose group.	on body weight, food consumption, haematology, and clinical chemistry; these signs are related to general toxicity of higher doses as generally seen in toxicology studies. However, a case by case approach may be done, as toxic adverse effects were not observed in all studies.		
4	Systemic toxicity	Body weight	rat	13 wk	Oral	25'000	ppm	Decrease	-22%/-11% (m/f) decrease at the highest dose.			
5	Systemic toxicity	Body weight	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				
7	Systemic toxicity	Body weight	rat	3 wk	Dermal	>1000	mg/kg bw/day	No effect				
8	Systemic toxicity	Body weight	mouse	4 wk	Dermal	>55.5	mg/0.1mL	No effect				
9	Systemic toxicity	Body weight	rat	2 yr	Oral	>20'000	ppm	Decrease				
10	Systemic toxicity	Body weight	rat	2 yr	Oral	8000/10'000	ppm	Decrease	-11% decrease in body weight gain at the highest dose in males and females. Decrease of 9% and 7.7% in the body weight in males and females, respectively.			
11	Systemic toxicity	Body weight	rat	91 wk	Oral	12'500 ppm; 531 mg/kg/d	ppm	Decrease	-12%			
12	Systemic toxicity	Body weight	mouse	2 yr	Oral	500	mg/kg bw/day	Decrease	27% decrease in body weight gain in males at the highest dose; and 25% and 38% in females at the two highest doses. Decrease in body weight of 12.8% in males of the 1000 mg/kg/day;			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									and decrease of 13% and 20% in the females of the two highest doses.			
13	Systemic toxicity	Body weight	mouse	102 wk	Dermal	55,5	other	Decrease				
14a	Systemic toxicity	Body weight	rat	15/10 (P/F1) wk	Oral	457	mg/kg bw/day	Decrease	Decrease in body weights at the highest dose of 457 mg/kg/day in pre mating periods in P males (7%) and F1 in males (12.2%) and females (10.7%). Decrease BW gain in P animals (23% and 24.4% in males and females, respectively) and F1 (13% and 20% in males and females, respectively). Decreases in body weight in females in GD0 (7% and 10% in the two control F0 dams; and 8% and 9% in the two control F1 dams); GD6 (4% and 8% in the two control F0 dams; and 3%			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									and 7% in the two control F1 dams); and GD13 (9% and 8% in the two control F1 dams). Decreases in body weight in females during in LD4 and LD7 in one of the F0 control groups (7% and 6%, respectively); and decreases in F1 LD0 controls (6% and 8% in both controls); LD4 (10% and 11%); LD7 (6% and 8%) and LD14 (8% in the second control group). The second F1 control group also showed and increase BWG during lactating period of 120%.			
14b	Systemic toxicity	Body weight	rat	15/10 (P/F1) wk	Oral	457	mg/kg bw/day	Decrease	Decrease in body weights at the highest dose of 457 mg/kg/day in F1B litters at day 21 (18%); F2B litters (12%); and F2A litters in days 14 and 21 (7% and 12%,			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									respectively).			
15	Systemic toxicity	Body weight	rat	10 wk	Oral	458	mg/kg bw/day	Decrease	At the highest dose of 458 mg/kg/day, decreased body weight throughout the experiment F1 females (9%), and F1 males (11%). Decrease in P females (7%) from day 21. During gestation (5-7%) and lactating days (5-7%) decreases at al measured days in F0 and F1.			
16	Systemic toxicity	Body weight	rat	GD 6-15	Oral	700	mg/kg bw/day	Decrease	At the dose of 700 mg/kg/day, decrased weight on GD 10 of 5.6% and on GD 16 of 5.7%. Body weight gain was decreased between days 6-9 (35%)			
17	Systemic toxicity	Body weight	rat	GD 6-15	Oral	300	mg/kg bw/day	Decrease	At the dose of 300 mg/kg/day, decreases in BWG at GD9: 17%; at GD 12: 18%; at GD 15: 28%; at GD 20: 20%. At the dose			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									of 600 mg/kg/day, decreases in BWG at GD9: 60%; at GD 12: 51%; at GD 15: 62% of controls; at GD 20: 46% (BW not measured).			
18	Systemic toxicity	Body weight	rabbit	GD 7-19	Oral	>500	mg/kg bw/day	Decrease	At the dose of 750 mg/kg/day reduced body weight on GD13 (19%) and GD16 (29%).			
19	Systemic toxicity	Body weight	rabbit	GD 7-19	Oral	250	mg/kg bw/day	No effect				
20	Systemic toxicity	Body weight	mouse	GD 7-15	Oral	1740	mg/kg bw/day	Decrease	Decreased body weight at all doses both in males (4%; 5%; 20%, at 1450; 1740; and 2100 mg/kg/day respectively) and females (8%; 4%; 20%, at 1450; 1740; and 2100 mg/kg/day respectively).			
28c	Systemic toxicity	Body weight	rat	PND 19-22	Oral	1000	mg/kg bw/day	Decrease	BW gain: 75% of controls at day 4. No difference in BW.			
29	Systemic toxicity	Body weight	rat	10 d	Oral	1000	mg/kg bw/day	Decrease	BW gain: 59% of controls (no statistically			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									significant)			
30	Systemic toxicity	Body weight	rat	10 d	Oral	1000	mg/kg bw/day	Decrease	BW gain: 73% of controls (no statistically significant)			
31	Systemic toxicity	Body weight	rat	PND 22-42	Oral	>900	mg/kg bw/day	No effect	BW gain: -12.9% between PND 22-35 (no statistically significant); no difference at the end of the experiment (PND42). No difference in BW.			
32	Systemic toxicity	Body weight	rat	PND 23-53	Oral	900	mg/kg bw/day	Decrease	-11.6% in body weight and -12,6% in body weight gain in the highest dose group.			
3	Systemic toxicity	Clinical chemistry and haematology	rat	3 mo	Oral	>20'000	ppm	No effect	Normal BUN levels			
4	Systemic toxicity	Clinical chemistry and haematology	rat	13 wk	Oral	12'500	ppm	Increase	1.25% Females: significantly reduced Hb and MCH; 2.5% Females: significantly reduced Hb and MCH. Males: significantly reduced RBC, Hb and MCHC.			
5	Systemic toxicity	Clinical chemistry and haematology	dog	1 yr	Oral	>300	mg/kg bw/day	No effect				

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
6	Systemic toxicity	Clinical chemistry and haematology	dog	1 yr	Oral	500	mg/kg bw/day	No effect				
7	Systemic toxicity	Clinical chemistry and haematology	rat	3 wk	Dermal	>1000	mg/kg bw/day	No effect				
10	Systemic toxicity	Clinical chemistry and haematology	rat	2 yr	Oral	>8000/10'000	ppm	Change	Increase in BUN (27%) in females at the highest dose and decrease of triglycerides (56%). In males increase in ALP (35% at the highest dose). In males decrease in triglycerides (44% and 61%, respectively at the two highest doses) and cholesterol (36% and 51% at the two highest doses). Decrease of proteins in urine in males (23% and 75% at the two highest doses, respectively) and in females (50% and 86% at the two highest doses, respectively). However, no confirmation of OPP-induced clinical chemistry			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									or hemathology changes in this study in either sex at any dose tested.			
12	Systemic toxicity	Clinical chemistry and haematology	mouse	2 yr	Oral	500	mg/kg bw/day	No effect				
31	Systemic toxicity	Clinical chemistry and haematology	rat	PND 22-42	Oral	900	mg/kg bw/day	Induction	Alanine aminotransferase (+102%), blood urea nitrogen (+23%), and phosphorus (+14%) levels were increased at 900 mg/kg/day			
32	Systemic toxicity	Clinical chemistry and haematology	rat	PND 23-53	Oral	900	mg/kg bw/day	Increase	Animals given 900 mg/kg/day had statistically-identified increase (27%) in BUN concentration; increases in serum ALT (95%) and AST (32%) activities.			
5	Systemic toxicity	Clinical signs	dog	1 yr	Oral	300	mg/kg bw/day	Increase	emesis after treatment at the dose of 300 mg/kg/day			
18	Systemic toxicity	Clinical signs	rabbit	GD 7-19	Oral	250	mg/kg bw/day	Increase	soft faeces and perineal soiling			
19	Systemic toxicity	Clinical signs	rabbit	GD 7-19	Oral	250	mg/kg bw/day	Increase	decreased faeces, decreased activity, perineal soiling, blood in pan			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
28b	Systemic toxicity	Clinical signs	rat	PND 19-22	Oral	>1000	mg/kg bw/day	No effect				
29	Systemic toxicity	Clinical signs	rat	10 d	Oral	1000	mg/kg bw/day	Change	In the highest dose animals, decreased activity, noisy respiration, clear or red perioral soiling, perineal soiling (urine and/or feces), and soft feces were observed. In the last period (days 7-11), 2 animals showed noisy respiration and a thir animal had perioral (clear) soiling.			
30	Systemic toxicity	Clinical signs	rat	10 d	Oral	1000	mg/kg bw/day	Change	In the highest dose group, one animal (excluded from the study) showed decreased activity; noisy respiration; perioral (clear) soiling; slow respiration; labored respiration; perineal (urine) soiling. Another animal showed perioral (clear) soiling; slow respiration;			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									decreased activity; perineal (urine) soiling; perinasal (red) soiling. And a third animal showed Noisy respiration; perioral (clear) soiling.			
32	Systemic toxicity	Clinical signs	rat	PND 23-53	Oral	>900	mg/kg bw/day	No effect				
4	Systemic toxicity	Food consumption	rat	13 wk	Oral	25'000	ppm	Decrease				
11	Systemic toxicity	Food consumption	rat	91 wk	Oral	25'000 ppm; 1140 mg/kg/d	ppm	Decrease	At the highest dose, significantly reduced food intake (g/rat). Increased relative food intake (g/kg bw/day)			
29	Systemic toxicity	Food consumption	rat	10 d	Oral	1000	mg/kg bw/day	Decrease	Day 4-7: 58% of controls. In the final period (7-11) no difference was observed.			
30	Systemic toxicity	Food consumption	rat	10 d	Oral	1000	mg/kg bw/day	Decrease	Day 4-7: 58% of controls (no statistically different in the last period 7-11)			
4	Systemic toxicity	Mortality	rat	13 wk	Oral	25'000	ppm	Increase	2 males and 1 female of the highest dose group died			
10	Systemic toxicity	Mortality	rat	2 yr	Oral	8000/10'000	ppm	Increase	Increase in mortality of the highest dose			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
									group (402 mg/kg/day) in males: 19 in control vs 24 in this group.			
11	Systemic toxicity	Mortality	rat	91 wk	Oral	12'500 ppm; 531 mg/kg/d	ppm	Increase	Survival: 71% vs 96% (highest dose vs control)			
17	Systemic toxicity	Mortality	rat	GD 6-15	Oral	1200	mg/kg bw/day	Increase	10/11 dams died after 3-9 days of treatment at the dose of 1200 mg/kg/day			
18	Systemic toxicity	Mortality	rabbit	GD 7-19	Oral	500	mg/kg bw/day	Increase	2/7 at 500 mg/kg/d and 6/7 at 750 mg/kg/d (deposition of test material in lungs). Due to the high rate of mortality, only one litter containing two embryos undergoing resorption was available in the 750 mg/kg group.			
19	Systemic toxicity	Mortality	rabbit	GD 7-19	Oral	250	mg/kg bw/day	Increase	At the 250 mg/kg/day group, 4/24 treatment-related deaths.			
20	Systemic toxicity	Mortality	mouse	GD 7-15	Oral	1450	mg/kg bw/day	Increase	4; 5; and 16 death females in the groups of 1450; 1740; and 2100 mg/kg/day, respectively.			

Study ID Matrix	Grouping	Lines of Evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
28a	Systemic toxicity	Mortality	rat	PND 19-22	Oral	>1000	mg/kg bw/day	No effect				
29	Systemic toxicity	Mortality	rat	10 d	Oral	>1000	mg/kg bw/day	No effect				
30	Systemic toxicity	Mortality	rat	10 d	Oral	>1000	mg/kg bw/day	No effect				
32	Systemic toxicity	Mortality	rat	PND 23-53	Oral	>900	mg/kg bw/day	No effect				

2.2.2.1 Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

Table 2.10.2.2.2.1/1: WoE for EAS-mediated adversity

- Regularly cycling was altered at 900 mg/kg/day (dose above MTD) in study ID 31 in rat. No information in mice or dogs is available. Oestrous cyclicity was not affected in studies ID 14 (two generation) and ID 15 (10 weeks of duration) in rat at a maximum dose of 457 mg/kg/day. However, some deviations were noted in these studies.

- Cervix histopathology was not altered in rat (ID 10, 14, 15 and 31). In mice, in study ID 13 (55.5 mg/0.1 mL, dermal exposure for 102 weeks) one female showed fibroma. No alteration was seen when OPP was administered orally route (ID 12).

- Mammary gland histopathology was only altered in females in study ID 12 (only one mouse presented anomalies at a dose of 1000 mg/kg/day). No incidences were seen in dogs or rats, nor in males in studies ID 5, 10, 12, in which this parameter was analysed. Oviduct histopathology was also altered in ID 12 study at the same dose in the presence of body weight losses (20%).

- Ovary histopathology was altered in the 2-year dermal study in mice (ID 13, exposure of 55.5 mg/0.1 mL), where there was an increase in the incidence of follicular cyst and luteoma; and in the study in rat (ID 31), in which one female presented juvenile appearance of the ovary (dose near or above MTD). As for ovary weight, +33% increase in relative ovary weight in P females in study ID 14 was observed. This may be attributed to unusually low control ovary weight in F0 females.

- Uterus histopathology varied only in study ID 31, in which two rats given 900 mg/kg/day had very slight decreased size of the uterus (B.W. of these animals were less than 17-19% of the mean group). In studies ID 4, 5, 6, 10, 12, 13, 14 and 15 no alterations were observed. In studies ID 4 (rat, 13 weeks) and 5 (dog, 1 year) uterus weight (the only studies which assessed this parameter) was neither altered.

- Vagina histopathology was only altered in the study ID 12 (2-year oral dosage in mouse), from the dose of 500 mg/kg/day (decreases in body weight of 12% and body weight gain of 25%). No alterations were observed in studies ID 5, 10, 14, 15, and 31.

- Coagulating gland histopathology was not altered in studies ID 12 (mouse, 2 year) nor ID 15 (rat, 10 weeks).

- Epididymis histopathology was not altered in dog or mouse, but variations were seen in the juvenile study in rats (ID 32), where immature and decreased spermatic elements were noted in the right epididymis of some control and treated animals at a dose above MTD. In this study, epididymis weight was decreased.

- Prostate histopathology was not altered in studies ID 4, 5, 10, 12, 13, 14 and 15 in rats, mice, and dogs. Nevertheless, ventral prostate and dorsolateral prostate showed marked weight decreases in study ID 32 in rats treated PND 23-53 at the dose of 900 mg/kg/day (above MTD).

- Seminal vesicles histopathology was not statistically significantly altered in any study, however in 2 generation reproduction study in rats (ID 14) there was an increase in secretion (at 457 mg/kg/day). In

studies ID 12 and 13 (in mice) there were no changes, nor in study ID 15 in rat (10 weeks at a maximum dose of 458 mg/kg/day). Seminal vesicles weight showed an increased weight in the only study it was measured (ID 32, at 900 mg/kg/day above the MTD).

- Testis histopathology was not altered in 1-year dog studies (ID 5 and 6). In 91-week oral study in rat (ID 11) at the dose of 1140 mg/kg/day, there was an increase in interstitial cell tumours of the testes. In this group of animals, there was an increase in mortality and a decrease in body weight. In the dermal 102-week carcinogenic study in mice (ID 13), there was an incidence of 1 interstitial cell tumour and 1 adenoma (55.5 mg/0.1 mL). Also, in mice, chronic/carcinogenic (ID 12) two rats treated with 1000 mg/kg/day presented Leydig cell tumours vs 1 in the control (not statistically analysed, presence of body weight losses of 5-10%). In rat oral 3 months (ID 3), 13 weeks (ID 4), 2 year (ID 10), no effects were observed, neither in rat two generation reproductive studies (ID 14 and 15) up to a dose of 458 mg/kg/day.

- Testis weight was altered in all studies in which this parameter was measured except in dog 1 year (ID 5) and rat 3 weeks dermal studies (ID 7). In rats, in study ID 4, a +20% increase in testis relative weight at the dose of 25'000 ppm was observed (decreased body weight of 22%); in ID 9, 46% increase in relative testis weight at 20'000 ppm; in ID 10, an increase of 34% of testes absolute weight in the 402 mg/kg/day group (8000 ppm) and an increase of 46% in the relative weight (in the presence of a decrease of body weight of 9% and body weight gain of 11%); in ID 14, increase relative testis weight (49%) at the dose of 457 mg/kg/day in F1 males; in ID 15, elevated relative testes weights in the high-dose group F1 males (12% above the control group). In juvenile study in male rat (ID 32), decreased adjusted and unadjusted weight of left testis (7% and 9%, respectively) at the highest dose of 900 mg/kg/day (above MTD). Right testis did not vary.
  In mouse in ID 12, increase in 14% of testes relative weight at the dose of 1000 mg/kg/day (decreases in body weight, 13%, and body weight gain, 27%).

- Pituitary weight was only analysed in 3 studies: ID 5, in which no alterations were observed (dog were dosed only given 5 days per week and emesis was observed at all doses); and in studies ID 31 and 32 (in rats), in which there was a decrease in relative (9.8%); and absolute (15%) and adjusted (11%) weight, respectively. In study ID 32 a male of the 900 mg/kg/day group presented pale pituitary (at these studies MTD was surpassed).

- Adrenal gland histopathology was only altered in mouse 102-week dermal study (dose of 55.5 mg/0.1 mL). Adrenal weight was increased in rat study ID 4 at a dose of 25'000 ppm (body weight decreases in males of 22% and in females of 11%); in mouse 2-year oral study ID 12 at a dose of 250 mg/kg/day (decreases in weight were observed at the doses of 500 and 1000 mg/kg/day); and in male pubertal study ID 32 from the dose of 250 mg/kg/day (systemic toxicity was only observed at the dose of 900 mg/kg/day). However, adrenal weight was decreased in studies ID 10 in female rats at the dose of 647 mg/kg/day and in PND 22-42 juvenile study in rat at the maximum dose of 900 mg/kg/day.

- Fertility index was decreased in study ID 20, mouse oral prenatal developmental, from the dose of 1450 mg/kg/day. No such alterations were seen in studies ID 14 in rat (457 mg/kg/day), 15 (where there was an increase at 458 mg/kg/day, attributed to the low values of control group) and ID 18 in rabbit (no effect at 500 mg/kg/day).

- Gestation length was not altered in the two generation reproduction toxicity tests (ID 14 and 15, up to a dose of 458 mg/kg/day) performed (rat).

- Litter size and litter viability were not altered in any of the prenatal development studies (ID 16, 18, 19, 20) in rats, mice, and rabbits. In study ID 17 in rats, a 6% decrease in males' weight and an 8.5% decrease in females were observed at the dose of 600 mg/kg/day, and a 27% decrease in males and females at the dose of 1200 mg/kg/day. In ID 20 in mice, the body weight of the live foetuses of both sexes was significantly reduced and a retardation of development was seen at 1450 mg/kg/day.
- Number or implantations was not altered in prenatal development studies 16, 18,19 and 20. In study ID 17 in rat, at the dose of 1200 mg/kg/day, a decrease of 8 vs 11.5 in control group was seen.
- The number of live births did not vary in two generation studies in rat (ID 14 and 15) nor in prenatal developmental studies in rat (ID 16 and 17).
- Post implantation loss was increased in rat study ID 17 at the doses of 600 (25.7%) and 1200 (38.5%) mg/kg/day vs the control group (13.9%). No alteration in other prenatal development studies was observed in rat, mouse or rabbit (ID 16, 18, 19, 20). Preimplantation loss was observed in study ID 20 in mouse (not specified). In rabbit studies no changes were seen.
- Some kind of anomalies were observed in prenatal developmental studies in rat (ID 16 and 17) and mouse (ID 20).
- Pup survival index (studies ID 14 and 15), sex ratio (ID 14, 15, 16, 19, 20) and time to mating (14 and 15) were not altered.
- Alterations in kidney histopathology (ID 4, 9, 10, 12, 15, 18, 19, 31, 32, from doses of 250 mg/kg/day), and in kidney weight (ID 4, 6, 10, 12, 14, 18, 19) were also observed.
- Alterations in liver histopathology were observed in studies ID 12, 15, 18 from doses of 250 mg/kg/day, and in liver weight in studies ID 3, 4, 10, 12, 14, 16, 31 and 32.

Table 2.10.2.2.2.1/2: WoE for EAS-mediated endocrine activity

- ER binding assay (ID 23) and ToxCast pathway model for agonist binding were classified as equivocal. CERAPP Potency Level (Consensus) indicate that OPP is a weak agonist and binder, and a very weak antagonist of ER.
- AR binding assay (ID 25) suggests that OPP can bind this receptor.
- OPP is classified as an inhibitor in the Aromatase Assay (ID 26).
- In the steroidogenesis assay (ID 27), the presence of OPP results in an increase of estradiol synthesis. On the other hand, the relationship is categorized as equivocal for testosterone.
- In the uterotrophic assay (ID 28) no alteration in uterus weight was observed up to the dose of 1000

mg/kg/day.

- In the antiandrogenic part of the Hershberger assay (ID 30) all measured tissues decreased their weights. However, it was only significant for ventral prostate (28%), which is especially sensitive to alterations in 5α-reductase. Seminal vesicles decreased their weight 12%; LABC 4%; glans penis 11%; and Cowper's glans 10%. On the other hand, there was a decrease in body weight gain of 27% (not statistically significant), as well as a no-significant increase in liver weight of 16%. Adrenals weight were no-significantly decreased 17%.

- In the female pubertal assay (ID 31) there was a decrease in the females regularly cycling at the highest dose (this tendency is also observed at the dose of 250 mg/kg/day). Age at first oestrus could only be determined for 10 of 14 animals in the 900 mg/kg/day group, because four animals did not have oestrus during the monitoring period. The mean age at first oestrus was PND 34.3 in controls, compared with PND 34.4, 35.1, and 33.0 in the 50, 250, and 900 mg/kg/day. However, despite significant body weight or body weight gain differences were not observed, some clinical chemistry parameters were altered (BUN was increased 23%). In addition, kidney histopathology showed alterations at the maximum dose of 900 mg/kg/day, including necrosis; therefore, it is highly possible that the MTD was exceeded.

- In ID 31, two rats given 900 mg/kg/day had very slight decreased size of the uterus (B.W of these animals were less than 17-19% of the mean group).

- In the male pubertal assay (ID 32) unadjusted age of balanopreputial separation was significantly increased (45.2 vs 43.1) at the dose of 900 mg/kg/day (adjusted BPS was done to PND 23 and not to PND 21, as it is stated in the guideline). In addition, weights of seminal vesicles plus coagulating gland with fluid, ventral prostate, LABC, left testis and left and right epididymis were decreased significantly at the dose of 900 mg/kg/day. However MTD was exceeded, based on decreased body weights (-11.6%), body weight gain (-12.6%), increased liver weights (+21% in relative liver weight and increase of adjusted (for PND 23) weight of 10%), increased BUN (27%) and kidney histopathology.

- Testis histopathology (ID 32) did not show any alteration, however, analysis was performed on right teste, while it had been left teste which had shown alteration in its weight. Immature and decreased spermatic elements were noted in the right epididymis of some control and treated animals.

- In study ID 32, no significantly alterations in testosterone serum levels were observed.

- In study ID 32, pituitary absolute and adjusted weight (PND23) at the highest dose was decreased (-15% and -11%, respectively). In study ID 31, pituitary showed a decrease of 9.8% less relative weight.

- Decreased adjusted adrenal weight (-11%) in females (ID 31) and increased absolute (16%) and adjusted (11%) weight in males (ID 32) at the dose of 900 mg/kg/day. No histological changes were observed.

In the guideline of the Hershberger assay (OECD 441), it is stated that to confirm endocrine activity, a test chemical should induce statistical changes in at least two tissues. However, it is recognized that antiandrogenic chemicals can act either as androgen receptor antagonists or 5α-reductase inhibitors. 5α-reductase inhibitors have a variable effect because conversion to dihydrotestosterone varies by tissue. Antiandrogens that inhibit 5α-reductase have more pronounced effects in ventral prostate than other tissues. This difference in tissue response

can be used to differentiate between AR mediated and 5α-reductase mediated modes of action. Therefore, the outcomes observed in study ID 30 should be taken into account.

It is remarkable that despite in the uterotrophic assay (ID 28) no alterations were observed, an antiestrogenic evaluation was not performed.

Regarding, pubertal female and male assays in rat (ID: 31 and 32, respectively), they present a questionable choice of doses, with a highest dose above the MTD and a second highest dose too low, contrary to the stated in the US EPA methods 890.1450 and 890.1500.

In study ID 31, at the maximum dose, in the female rat study, there were alterations in the regularity of oestrus cycle (which in the guideline is considered more important than a lack of statistical significance for the difference in weight of ovary or uterus in treated animals). In addition, age at first oestrus could only be determined for 10 of 14 animals. However, abnormal blood chemistry values, were found. BUN was increased (+23%) which may indicate, even in the absence of effects in body weight and body weight gain, that the MTD was exceeded. Alanine aminotransferase (+102%) and phosphorus (+14%) were also significantly altered. Effects on relative liver weight and kidney histopathology, including necrosis, were also observed. Therefore, MTD may have been reached.

In pubertal male rat assay (ID 32), toxic effects were observed in the animals treated with 900 mg/kg/day (increased relative (21%) and adjusted (10%) liver weight, increased BUN concentration (27%) and decreased body weight (11.6%) and body weight gain (12.6%)). In the followed guideline it is stated that studies that suggest interaction with the endocrine system only at a dose level causing more than approximately 6% decrease in body weight gain at termination compared to controls may require additional studies and/or a weight-of-evidence approach using other information in order to be interpretable. Consequently, the observed effects in accessory sex tissue and reproductive organ weight (statistically significant decrease of seminal vesicles plus coagulating gland fluid, ventral prostate, LABC, lefts testis and epididymis) as well as the delay of the age of balanopreputial separation, cannot be carelessly regarded to draw a conclusion on endocrine disruption effects. Nevertheless, the lack of effects at the dose of 250 mg/kg/day is neither considered relevant to confirm the absence of endocrine disruption adversity, since this dose is too low. In addition, adjustment of measured parameters was done to PND 23 and not PND21, as it is stated in the US EPA 890.1500 guideline.

It should be also noted that the developmental and reproductive studies were conducted according to outdated versions of their guidelines, and their outcomes are debatable due to some deviations noted in their methodology and/or in the analysis of the results. As it is highlighted in Kwok and Silva (2013) (B.6.6.2-06), there are circumstances that do not allow to extract fully trustworthy conclusions.

- In the study ID 17 (B.6.6.2-01), the foetus (not the litter) was the experimental unit for the statistical analysis of resorptions and therefore, the increased resorption in OPP-treated dams may be equivocal. In addition, study authors did not describe their methods for measuring “fertility.”
- In the study ID 16 (B.6.6.2-02), results were not recorded for two control dams and four dams at 700 mg/kg/day because they were given the wrong dose, were not pregnant, or delivered early. In addition, only 1/3 of the foetuses in each treatment group were examined for external or visceral effects. Skeletal examinations were performed on all foetuses and three skeletal anomalies were statistically significantly increased (~13-15%) at 700 mg/kg/day (delayed ossification of sternebrae, pinpoint holes in the occipital or interparietal plates in the skull, and skull bone island). Delayed ossification in the sternebrae was observed in 3% of foetuses and 30% of litters at 700 mg/kg/day and was outside the historical controls (5% foetuses and 28% litters). Pinpoint holes in the occipital or interparietal plates in the skull increased at ≥ 300 mg/kg/day and bone-island was increased at all doses. Historical controls for these effects in the skull was 0/2320 litters (MARTA, 1996)[9]. Uteri from animals that did not appear to be pregnant were stained with 10% solution of sodium sulfide. This procedure was performed only to test for implantation sites, and a different procedure (not explained) was used to determine foetal resorptions. In the study, pre-implantation loss was calculated as a proportion of the numbers of corpora lutea not associated with implantation. The report did not subsequently address this effect, although the analysis of the data performed in the Kwok and Silva study, indicated a statistically significant ($p<0.05$) increase in pre-implantation loss at 700 mg/kg/day. The analysis was performed using the percent pre-implantation loss per litter as an experimental unit and nonparametric (i.e., distribution free) tests for multiple comparison (Williams 1972[10], 1986[11]). The occurrence of pre-implantation loss (16/34 (47%); 15/25 (60%); 17/26

[9] MARTA (1996) Historical Control Data (1992 — 1994) for Developmental and Reproductive Toxicity Studies using the Crl:CD®(SD)BR Rat.

[10] Williams, D.A. (1972) The comparison of several dose levels with a zero dose control. Biometrics 28: 519-531.

11 Williams, D.A. (1986) A note on Shirley’s nonparametric test for comparing several dose levels with a zero-dose control. Biometrics 42: 183-186.

(65%); 15/20 (75%), at the control; 100; 300; 700 mg/kg/day groups, respectively) is an unexpected finding because treatments started after implantation had occurred. Because resorptions detected only by sodium sulfide staining were not counted toward total resorptions, it is possible that some of the instances of pre-implantation loss at 700 mg/kg/day might be instances of early resorption (i.e., post-implantation loss). However, historical control data from the conducting laboratory are unavailable for further evaluating the biological significance of this finding.

- In the study ID 20 (B.6.6.2-05), the numbers of corpora lutea per dam were comparable among the four groups; however the decreases in the numbers of implantation sites per dam at 200 and 400 mg/kg/day were consistent with pre-implantation loss. As with study ID 16, treatments commenced on GD 7, which was after the interval that implantations occur in the mouse (GD 4.5-5) (Brinster. 1975)[12]. The apparent pre-implantation loss might reflect early post-implantation loss that went unrecognized in the study (staining methods are not described).
- In the study ID 18 (B.6.6.2-03), the report did not describe the uterine contents, except to indicate that the animal was pregnant. 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/day, respectively. The report did not provide data for foetal examinations.
- In the study ID 19 (B.6.6.2-04), the only developmental effect of OPP in rabbits was an increased incidence of litters with resorptions. However, it may have been dismissed the possible effect of resorptions (statistically significant increase in resorptions was not found). The statistical method employed was censored Wilcoxon test for pairwise comparison (Haseman, 1974)[13] with a Bonferroni correction for controlling Type I error and the number of affected foetuses per litter as an experimental unit.

  The analyses performed by Kwok and Silva (2013) indicate that the dismissal of the possible toxicological significance of the reported resorptions may not be appropriate. For evaluating discrete-response variables like resorptions in a developmental toxicity study, Haseman and Piegorsch (1994)[14] recommended that the statistical analysis should be based on proportion of affected foetuses instead of the number affected foetuses; the latter metric gives no consideration to the potential effect of the test chemical on litter size. Also, in an article by Haseman *et al*., 2001[15], concern was raised regarding the application of Bonferroni correction to the p-values when making pairwise comparison due to a relatively high false-negative rate. These authors suggested that Bonferroni correction would be unnecessary if multiple comparison procedures were used.

  The reanalysis of the resorptions by Kwok and Silva (2013) in OPP-treated rabbits using the percent resorptions per litter as an experimental unit and nonparametric (i.e., distribution free) tests for dose response (Jonckheere, 1954[16]; Lehman and D'Abrera, 1975[17]) and multiple comparison (Williams 1972[18], 1986[19]) found that resorptions exhibited a significant ($p<0.05$) dose-related trend and were significantly ($p<0.05$) increased at 100 and 250 mg/kg/day (Vol. 3, Table B.6.6.2-06/3). Likewise, analysis of the combined data from both phases (following the approach of study ID 18) indicates a statistically significant increase in effects at 100 and 250 mg/kg/day (Vol. 3, Table B.6.6.2-06/3).

12 Brinster, R.L. (1975) Teratogen testing using preimplantation mammalian embryos. In: Miller, JR, Marois, M, Shepard, TH (eds.) Methods for detection of environmental agents that produce congenital defects: proceedings of the Guadeloupe Conference Sponsored by 1'Institut de la Vie,. North-Holland Pub. Co.; American Elsevier Pub. Co., Amsterdam, New York 113-124.

13 Haseman, J.K., Hoel, D.G. (1974) Tables of gehan's generalized Wilcoxon test with fixed point censoring. Journal of Statistical Computation and Simulation 3: 117 - 135.

14 Haseman, J.K., Piegorsch, W.W. (1994) Statistical Analysis of Developmental Toxicity Data. In: Kimmel, CA, Buelke-Sam, J (eds.) Developmental toxicology, 2nd ed edn. Raven Press, New York 349-362.

15 Haseman, J.K., Bailer, A.J., Kodell, R.L., Morris, R., Portier, K. (2001) Statistical issues in the analysis of low-dose endocrine disruptor data. Toxicolological Sciences 61: 201-210.

16 Jonckheere, A.R. (1954) A distribution-free k-sample test against ordered alternatives Biometrika 41: 133-145.

17 Lehmann, E.L., D'Abrera, H.J.M. (1975) Nonparametrics: statistical methods based on ranks, San Francisco Holden-Day.

18 Williams, D.A. (1972) The comparison of several dose levels with a zero dose control. Biometrics 28: 519-531.

19 Williams, D.A. (1986) A note on Shirley's nonparametric test for comparing several dose levels with a zero-dose control. Biometrics 42: 183-186.

Table 2.10.2.2.2.1/3: (Vol. 3 Table B.6.6.2-06/3) Ocurrence of litters with resorptions in a developmental-toxicity study of OPP using New Zealand White rabbits

Litters[a]	mg/kg/day					
	0		25	100	250	
	1st Phase	2nd Phase	1st Phase	1st Phase	1st Phase	2nd Phase
1	100[b]		100	60.0	100	
2	33.3		36.4	50.0		33.3
3		22.2	33.3	25.0		33.3
4	14.3		20.0	22.2	28.6	
5	12.5		14.3	20.0	25	
6	0[c]		11.1	20.0		20
7	0		9.1	16.7	16.7	
8	0		9.1	12.5	16.7	
9	0		0	12.5	14.3	
10	0		0	10	12.5	
11	0		0	0		11.1
12	0		0	0	9.1	
13	0		0	0	9.1	
14	0		0		0	
15		0			0	
16						0
17						0
18						0
First Phase Data Only						
Litter incidence	4/13 (31%)		8/14 (57%)	10/13 (77%)	9/11 (82%)	
Percent post-implantation loss[d]	12.3 ± 28.1[e]		16.7 ± 26.9	19.2 ± 18.1[e]	21.1 ± 27.6[e]	
Combined Data						
Litter incidence	5/15 (33%)		8/14 (57%)	10/13 (77%)	13/18 (72%)	
Percent post-implantation loss[d]	12.2 ± 26.4[e]		16.7 ± 26.9	19.2 ± 18.1[e]	18.3 ± 23.3[e][f]	

Abbreviations: NS: not significant. Shading identifies data from the second phase of testing.
[a] In columns 2-6, litters are presented in an ordered fashion. The first column only provides a visual aid for showing the number of litters per group.
[b] Percent implantations that were resorptions in a litter; e.g., 100% means that all of the implantations were resorptions.
[c] Litter with no resorptions.
[d] Percent post-implantation loss is the sum of percent resorptions per litter divided by the total number of litters.
[e] Nonparametric (i.e., distribution free) ranked-based trend test for ordered alternatives [24, 30] with the percent affected per litter as an experimental unit [20], significant at p≤0.05.
[f] Non-parametric multiple-comparison test [55, 56] with the percent affected per litter as an experimental unit [20], significant at p≤0.05.
[g] Calculated t-value (1.68) was comparable to the table value of 1.72 at α=0.05 [55].

Historical control data for percent litters with resorptions in the conducting laboratory were submitted by the investigators Breslin *et al*., 1992[20], (Vol. 3, Table B.6.6.2-06/2) and applied to the calculations. From Vol. 3, Table B.6.6.2-06/3, the percent litters with resorptions (i.e., incidence of resorptions) in the first phase for the 0, 25, 100, and 250 mg/kg/day groups were 31%, 57%, 77%, and 82%, respectively. The resorptions at 100 and 250 mg/kg/day were double that were observed in the concurrent controls and clearly exceeded the historical control range (i.e., 66.7%).

Carney and Zablonty (2006)[21] in the re-evaluation of the study acknowledged that the percent postimplantation loss was slightly (but not statistically significantly) higher than the controls. However, there were no details on how the statistical analysis was performed (Vol. 3, Table B.6.6.2-06/2).

In addition, significant deviations from the Guidelines may contribute to the ostensible negative results in some studies.

- In the study ID 14 (B.6.6.1-01), there were deviations from the guideline protocol that may have affected mating results (e.g., dams were cohoused with a male for only 1-2 days per mating week). Given that the oestrus cycle in young rats is typically 4-5 days and that the cycle shifts to even longer durations with increasing age, the reason for cohousing for less than 4 days (i.e., less than one cycle) was not known.

20 Breslin, W.J., Kociba, R.J., Landenberger, B.D. (1992) Response to CDPR MT Record Number 097303: *ortho*-Phenylphenol (OPP) Gavage Teratology Study in New Zealand White Rabbits (Additional data to record number 97303 in Volume 129-0148). The Dow Chemical Company.

21 Carney E, Zablotny C (2006) Developmental toxicity endpoint. Response to Department of Pesticide Regulation *Ortho*-Phenylphenol (OPP) and Sodium *Ortho*-Phenylphenate (SOPP) Risk Characterization Document (RCD): Dietary Exposure Draft. Lanxess Corporation and The Dow Chemical Company 27-30.

Dams that were classified as having not mated in the study almost categorically had not been cohoused with a male for the 21- day minimum given in earlier FIFRA Guidelines or the 16- day minimum (4×4) indicated in the conducting laboratory's Standard Operating Procedures (SOP) (in the OECD 416 guideline the period is 2 weeks or until copulation occurs). In the case of 9 F0 dams, the total number of cohousing days was only 11-13. In 12 instances, dams were noted as having a sperm plug in their bedding or in one case in the dam's vagina (F1b dam) but these dams were not classified as having mated based on finding these plugs. It should be noted that the current and former FIFRA Guidelines (as well as in the 416 OECD guideline) specify that a plug is taken to be evidence of mating and that the day of its finding is used to define day 0 of the pregnancy. It was noted that dams possibly had sperm in their vaginal wash but were not designated as having mated and this may have affected the male fertility index. Consequently, it is considered that the assessments on fertility in this study were inconclusive.

- In the study ID 15 (B.6.6.1-02), the control- and low-dose fertility (number pregnant/number mated) and fecundity indices were low compared with those at the mid and high dose for the F1 (F1a mating) as were the fecundity indices (number of live deliveries/number mated) for the F1 (F2b mating). The fecundity indices at 500 mg/kg/day for F1 (F1a and F2a matings) were statistically significantly increased over controls. It is a concern that the least ability to procreate was seen in the controls of the F2a and F2b mating trials: fecundity indices for the controls were 0.5 (15/30) and 0.6 (18/30), respectively. A similar situation also occurred in the first reproduction study with the F1b control group: the dam fecundity index was only 0.23 (7/31). Adding to the concern is that the ability to procreate (as it is indicated by the fertility index) increased with increasing dose in two consecutive mating trials (F2a and F2b). When evaluating both the fecundity and fertility indices, it appeared that the control group did not function as would be expected, and then, the potential for identification of true effects induced by treatments is limited.

Therefore, outcomes from reproductive and developmental studies (ID 14-20) should be assessed very carefully.

2.2.3 Initial analysis of the evidence and identification of relevant scenario for the ED assessment of EAS-modalities

Table 2.10.2.2.3: Selection of relevant scenario

Adversity based on EAS-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "**EAS-mediated**" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	X
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no **EAS-mediated endocrine activity** observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.2.4 MoA analysis for EAS modalities

The weight of evidence indicates that changes in endocrine activity were consistently observed across different studies conducted at different doses and different lengths of treatment.

Several *in vitro* and *in vivo* mechanistic studies show any alteration: ER binding assay (B.6.8.3-02, equivocal result), AR binding assay (B.6.8.3-03, positive result), aromatase assay (B.6.8.3-04, inhibition of the enzyme), steroidogenesis (B.6.8.3-05, positive result), Hershberger assay (B.6.8.3-07, significantly alteration of ventral prostate weight) and pubertal assay in female and male rats (B.6.8.3-08, and B.6.8.3-09, respectively), where different types of alterations are observed, including oestrous cycle irregularities and delay of balanopreputial separation (at doses above MTD).

It should be noted that if the results in the pubertal male assay were at a lower dose (according to the US EPA guideline a deviation in the chosen doses is noted, as it is indicated in Vol. 3 study B.6.8.3-09), steroidogenesis inhibition or hypothalamic pituitary gonadal axis suppression may be considered due to the observed increased in the age of puberty and the decreases of all measured organ sex tissues.

Both antagonism of androgen receptor (seen in study ID 25) or the inhibition of 5α-reductase (as it may be extracted from study ID 30), can lead to altered perineal differentiation, short male AGD and feminized offspring. However, these parameters were not determined in any study. In addition, as it was previously mentioned, in the Hershberger assay only ventral prostate weight was altered, which may diminish the relevance of the finding.

However, in line with the importance of the 5α-reductase alteration, AOP pathway 288 (from AOP wiki) relates decreased dihydrotestosterone levels with decreased androgen receptor activation and posterior impaired inguinoscrotal testicular descent and cryptorchidism.

Cook *et al*., 1999[22], also related estrogen antagonism, androgen antagonism, aromatase inhibition or 5α-reductase inhibition with Leydig cell hyperplasia or adenoma.

Kwok and Silva (2013) (B.6.6.2-06) also proposed a potential MoA for the developmental effects. OPP was positive in several studies for endocrine disrupting potential *in vitro*[23][24][25][26][27]. The assay systems used were estrogen-receptor binding (non-competitive), estrogen-induced cell proliferation (e.g., MCF-7 human breast cancer cells), and estrogen-receptor transcription activity in cells (e.g., MVLN cell line). In addition, Freyberger and Degen[28] discovered that in ovine seminal vesicles, OPP as well as its metabolite PHQ were inhibitors of prostaglandin synthase. Habicht and Brune[29] determined an IC50 value of 2.5 µM for OPP inhibition of the release of prostaglandin E2 using phorbol ester stimulated mouse peritoneal macrophages in testing *in vitro*. Therefore, OPP and PHQ may be acting *in vivo* as inhibitors of prostaglandin metabolism. It should be noted that some inhibitors of prostaglandin (e.g., Nonsteroidal Anti-inflammatory Drugs) have been reported to increase resorptions in rats[30] [31]and rabbits[32] and to induce cleft palate in mice[33].

On the other hand, as it is seen in AOP 7 (from AOP wiki) an inhibition of the aromatase (as seen *in vitro* in study ID 26) can lead to ovarian cycle irregularities (observed in study ID 31, at the dose of 900 mg/kg/day), which is

22 Cook, J.C., Klinefelter, G.R., Hardisty, JF, Sharpe, R.M., Foster, P.M. (1999). Rodent Leydig cell tumorigenesis: A review of the physiology, pathology, mechanisms, and relevance to humans. Critical Reviews in Toxicology, 29, 169-261

23 Blair RM, Fang H, Branham WS, Hass BS, Dial SL, Moland CL, Tong W, Shi L, Perkins R, Sheehan DM (2000) The estrogen receptor relative binding affinities of 188 natural and xenochemicals: structural diversity of ligands. Toxicological Sciences 54: 138-153.

24 Miller D, Wheals BB, Beresford N, Sumpter JP (2001) Estrogenic activity of phenolic additives determined by an in vitro yeast bioassay. Environmental Health Perspective 109: 133-138.

25 Rehmann K, Schramm KW, Kettrup AA (1999) Applicability of a yeast oestrogen screen for the detection of oestrogen-like activities in environmental samples. Chemosphere 38: 3303-3312.

2626 Routledge EJ, Sumpter JP (1997) Structural features of alkylphenolic chemicals associated with estrogenic activity. Journal of Biological Chemistry 272: 3280- 3288.

27 Soto AM, Fernandez MF, Luizzi MF, Oles Karasko AS, Sonnenschein C (1997) Developing a marker of exposure to xenoestrogen mixtures in human serum. Environmental Health Perspective 105 Suppl 3: 647-654.

28 Freyberger A, Degen GH (1998) Inhibition of prostaglandin-H-synthase by o-phenylphenol and its metabolites. Archives of Toxicology 72: 637-644.

29 Habicht J, Brune K (1983) Inhibition of prostaglandin E2 release by salicylates, benzoates and phenols: a quantitative structure-activity study. Journal of Pharmacy and Pharmacology 35: 718-723.

30 17. John JA, Murray FJ, Rao KS, Schwetz BA (1981) Teratological evaluation of *ortho*phenylphenol in rats. Fundamental and Applied Toxicology 1: 282-285.

31 MARTA (1996) Historical Control Data (1992 — 1994) for Developmental and Reproductive Toxicity Studies using the Crl:CD®(SD)BR Rat.

32 O'Grady JP, Caldwell BV, Auletta FJ, Speroff L (1972) The effects of an inhibitor of prostaglandin synthesis (indomethacin) on ovulation, pregnancy, and pseudopregnancy in the rabbit. Prostaglandins 1: 97-106.

33 Montenegro MA, Palomino H (1990) Induction of cleft palate in mice by inhibitors of prostaglandin synthesis. Journal of Craniafacial Genetics and Developmental Biology 10: 83-94.

also directly related to impaired fertility (observed in study ID 20 at the dose of 1450 mg/kg/day in mouse). Despite this concordance between one KE to the next in the sequence it is considered that to establish more reliable and quantitative linkages more information is required. As it was previously stated, the weight of results from the study ID 20 is questionable, and the dose of 900 mg/kg/day in study ID 31 may be above MTD. Oestrous cycle was also assessed in studies ID 14 and ID 15 without showing alterations; however, dose spacing and rest before second matings were not the indicated in the OECD 416 guideline.

Consequently, in light of these facts, it is considered that there is a lack of information on key parameters *in vivo*, which does not allow to perform a MoA.

In the following table, a time concordance for the observed EAS modalities related findings is shown:

Table 2.10.2.2.4: Dose and time concordance for EAS mediated effects

mg/kg/d	Species	Juvenile PND 22-42 (ID 31)	Juvenile PND 23-53 (ID 32)	GD 6-15 (ID 16, ID 17)	GD 7-15 (ID 20)	10 days (Hershberger) (ID 30)	13 weeks (ID 4)	91 weeks (ID 11)	102 weeks (ID 13)	2 years (ID 12)	2 years (ID 10)	2 generation reproductive (ID 14, ID 15)	2 years (rat) (ID 9)	ppm (rat)
55.5/0.1 mg/mL (dermal)	mouse								(↑) alterations in adrenal, ovary, cervix, and testes histopathology (↓) males body wt (5-10%) ID: 13				Rat: (↑) rel testes wt (46%) (↑) kidney histopathology alteration ID: 9	20000 (approx. 1000 to 2000 mg/kg bw/day)
248 (females)	rat										(↓) kidney abs wt (8%) (↓) liver abs wt (9.5%) ID 10			
250	mouse /rat		(↑) liver rel wt (8%) (↑) adrenal adj wt (9%) ID: 32, rat							(↑) adrenal rel wt (m) (16%) (↑) kidney histopathology alteration (m) (↑) kidney histopathology alteration (m/f) ID: 12, mouse				
391	rat						(↑) liver rel wt (7.3%) (m) ID: 4							

mg/kg/d	Species	Juvenile PND 22-42 (ID 31)	Juvenile PND 23-53 (ID 32)	GD 6-15 (ID 16, ID 17)	GD 7-15 (ID 20)	10 days (Hershberger) (ID 30)	13 weeks (ID 4)	91 weeks (ID 11)	102 weeks (ID 13)	2 years (ID 12)	2 years (ID 10)	2 generation reproductive (ID 14, ID 15)	2 years (rat) (ID 9)	ppm (rat)
402 (males)	rat										(↑) slight increase in mortality (↑) testis abs (34%) and rel wt (46%) (↓) bw (9%) and bwg (11%) ID: 10			
457	rat											(↓) litter wt F1 and F2 (↑) P females ovary rel wt (33%) (↑) testes rel wt in F1 (↑ ns) seminal vesicle secretion P males (↓) body wt (m/f) during premating, gestation and lactation (↓) liver wt (13.4%) F1 (f) (↑) kidney rel wt P (8%) and F1 (11%) ID: 14		
458	rat											(↓) pup wt F1 and F2 (>10%)		

mg/kg/d	Species	Juvenile PND 22-42 (ID 31)	Juvenile PND 23-53 (ID 32)	GD 6-15 (ID 16, ID 17)	GD 7-15 (ID 20)	10 days (Hershberger) (ID 30)	13 weeks (ID 4)	91 weeks (ID 11)	102 weeks (ID 13)	2 years (ID 12)	2 years (ID 10)	2 generation reproductive (ID 14, ID 15)	2 years (rat) (ID 9)	ppm (rat)
												(↑) testes rel wt (12%) (↑) fertility index in F2 (↓) bw P females; F1 m (11%)/f (9%) (↑) alterations in kidney histopath. ID 15		
500	mouse									(↑) adrenal rel wt (m) (18%) (↑ ns) vagina histopathology alteration (↑) brain rel wt (m/f) (10% and 15%) (↑) kidney histopathology alteration (m) (↑) kidney rel wt (17%) (f) (↓) kidney abs wt (7%) (m) (↑) liver histopathology				

mg/kg/d	Species	Juvenile PND 22-42 (ID 31)	Juvenile PND 23-53 (ID 32)	GD 6-15 (ID 16, ID 17)	GD 7-15 (ID 20)	10 days (Hershberger) (ID 30)	13 weeks (ID 4)	91 weeks (ID 11)	102 weeks (ID 13)	2 years (ID 12)	2 years (ID 10)	2 generation reproductive (ID 14, ID 15)	2 years (rat) (ID 9)	ppm (rat)
										alteration (m/f) (↓) bwg 25% and bw 13% bwt(f) ID: 12				
600	rat			(↑) post implantation loss (25.7%) (↓) litter wt (6% m/ 8.5% f) (↓ ndr) dams bwg ID: 17										
647 (females)	rat										(↓) adrenal rel wt (13.6%) (↑) kidney histopathology alteration (↓) kidney abs wt (11%) (↓) liver abs wt (12.5%) (↓) bwg (11%) ID: 10			
700	rat			(↓) skull ossification (↓) liver wt ID: 16										
761	rat						(↑) liver							

mg/kg/d	Species	Juvenile PND 22-42 (ID 31)	Juvenile PND 23-53 (ID 32)	GD 6-15 (ID 16, ID 17)	GD 7-15 (ID 20)	10 days (Hershberger) (ID 30)	13 weeks (ID 4)	91 weeks (ID 11)	102 weeks (ID 13)	2 years (ID 12)	2 years (ID 10)	2 generation reproductive (ID 14, ID 15)		2 years (rat) (ID 9)	ppm (rat)
							rel wt (7.3%) (m) (↑) kidney rel wt (4.3%) (m) ID: 4								
900	rat	(↑) females not cycling; (↑) irregular cycling; (ns) one rat juvenile appearing of ovary; (↓) size uterus in two rats (↓) pituitary rel wt (9.8%) (↓) adrenal adj wt (12.8%) (↑) liver rel wt (9.3%) (↑) BUN (23%) (↑) kidney histopathology alterations ID: 31	(↑) unadjusted age BPS (2.1 days) (↓) coag. gland w/fluid adj wt (19%) unadj wt (23%); coag. gland wo/fluid adj wt (14%) (↓) left testis wt adj (7%); unadj wt (9%) (↓) ventral prostate wt adj (17%); unadj wt (20%) (↓n.s.) dorsolateral prostate wt adj (11%); unadj wt (15%) (↓) LABC wt adj (16%); unadj wt (18%) (↓) left epididymis												

mg/kg/d	Species	Juvenile PND 22-42 (ID 31)	Juvenile PND 23-53 (ID 32)	GD 6-15 (ID 16, ID 17)	GD 7-15 (ID 20)	10 days (Hershberger) (ID 30)	13 weeks (ID 4)	91 weeks (ID 11)	102 weeks (ID 13)	2 years (ID 12)	2 years (ID 10)	2 generation reproductive (ID 14, ID 15)		2 years (rat) (ID 9)	ppm (rat)
			wt adj (6%); right epididymis adj wt (4%) (↑) immature spermatic elements (↓) bw (11.6%); (↓) bwg (12.6%) (↓) pituitary abs wt (15%) and adj wt (11%) (↑) liver rel wt (21%) and adj wt (10%) (↑) adrenal wt (16%) adj wt (11%) (↑) BUN (27%) (↑) kidney histopathology alterations ID: 32												
1000	mouse /rat					(↓) ventral prostate wt (28%) (↓ ns) seminal vesicles wt (12%) (↓ ns) LABC wt (4%) (↓ ns) glans				(↑) testes rel wt (12%) (↑ ns) Leydig cell tumour (↑ ns) ovary cyst (↑ ns)				

mg/kg/d	Species	Juvenile PND 22-42 (ID 31)	Juvenile PND 23-53 (ID 32)	GD 6-15 (ID 16, ID 17)	GD 7-15 (ID 20)	10 days (Hershberger) (ID 30)	13 weeks (ID 4)	91 weeks (ID 11)	102 weeks (ID 13)	2 years (ID 12)	2 years (ID 10)	2 generation reproductive (ID 14, ID 15)		2 years (rat) (ID 9)	ppm (rat)
						penis wt (11%) (↓ ns) Cowpers glands wt (10%) (↓ ns) adrenal wt (12.8%) (↑ ns) liver wt (16%) (↓ns) bwg (27%) ID: 30				mammary gland alteration (f) (↑ ns) vagina histopathology alteration (↑) kidney histopathology alteration (m) (↑) kidney rel wt (20%) (f) (↓) kidney abs wt (14%) (m) (↑) liver histopathology alteration (m/f) (↑ndr) liver rel wt (46%) (f); (↑ndr) liver abs wt (23%) (f); (↑) adrenal rel wt (m) (50%) abs wt (33%) (m) (↑) brain rel wt				

mg/kg/d	Species	Juvenile PND 22-42 (ID 31)	Juvenile PND 23-53 (ID 32)	GD 6-15 (ID 16, ID 17)	GD 7-15 (ID 20)	10 days (Hershberger) (ID 30)	13 weeks (ID 4)	91 weeks (ID 11)	102 weeks (ID 13)	2 years (ID 12)	2 years (ID 10)	2 generation reproductive (ID 14, ID 15)		2 years (rat) (ID 9)	ppm (rat)
										(m/f) (15% and 23%) (↓) bwg 27% (m), 38% (f) and bw 12.8% (m), 20% (f) ID: 12					
1140	rat							(↑) cell tumours in testes (ns) (↓) bodywt (12%) (↑) mortality (↑) kidney histopathology alteration ID: 11							
1200	rat			(↑) post implantation loss (38.5%) (↓) number of live births (↓) litter wt (27% m/f) (↑) mortality of dams											

mg/kg/d	Species	Juvenile PND 22-42 (ID 31)	Juvenile PND 23-53 (ID 32)	GD 6-15 (ID 16, ID 17)	GD 7-15 (ID 20)	10 days (Hershberger) (ID 30)	13 weeks (ID 4)	91 weeks (ID 11)	102 weeks (ID 13)	2 years (ID 12)	2 years (ID 10)	2 generation reproductive (ID 14, ID 15)		2 years (rat) (ID 9)	ppm (rat)
				(↓ ndr) dams bwg ID: 17											
1450	mouse				(↓) bw live fetuses of both sexes. Retardation of development (↓) fertility index (↓) parental bw (↑) mortality ID: 20										
1669	rat						(↑) liver rel wt (11%/13%) (m/f) (↑) kidney rel wt (5.7%) (m) ID: 4								
1740	mouse				(↓) bw live fetuses of both sexes. Retardation of development (↓) fertility index (↓)										

mg/kg/d	Species	Juvenile PND 22-42 (ID 31)	Juvenile PND 23-53 (ID 32)	GD 6-15 (ID 16, ID 17)	GD 7-15 (ID 20)	10 days (Hershberger) (ID 30)	13 weeks (ID 4)	91 weeks (ID 11)	102 weeks (ID 13)	2 years (ID 12)	2 years (ID 10)	2 generation reproductive (ID 14, ID 15)		2 years (rat) (ID 9)	ppm (rat)
					parental bw (↑) mortality ID: 20										
2100	mouse				(↓) bw live fetuses of both sexes. Retardation of development (↓) fertility index (↓) parental bw (↑) mortality ID: 20										
2978	rat						(↑) testes rel wt (20%) (↑) adrenal rel wt (22% and 9.8%) (m/f) (↑) liver rel wt (20%/33%) (m/f) (↑) liver abs wt (15%) (f) (↑) kidney								

mg/kg/d	Species	Juvenile PND 22-42 (ID 31)	Juvenile PND 23-53 (ID 32)	GD 6-15 (ID 16, ID 17)	GD 7-15 (ID 20)	10 days (Hershberger) (ID 30)	13 weeks (ID 4)	91 weeks (ID 11)	102 weeks (ID 13)	2 years (ID 12)	2 years (ID 10)	2 generation reproductive (ID 14, ID 15)		2 years (rat) (ID 9)	ppm (rat)
							rel wt (25% and 15%) (m/f) (↓) brain abs wt (5%) and rel wt (18%) (m) (↑) brain rel wt (10%) (f) (↑) mortality (↓) body wt (22%/11%) (m/f) ID: 4								

2.2.4.1 Postulate MoA

Based on the available information and on the lines of evidence described, it is not possible to fully describe a MoA, mainly due to the absence of evidences in level 4 or 5 studies, because they were not addressed; because effects were observed, but at too high doses (and not studied at lower doses); because there are not endocrine effects but in methodologically poor studies, or because adverse effects were not observed. Therefore, despite EAS activity has been observed, it has not been posible to confirm neither discard endocrine adversity.

2.2.4.2 Further information to be generated to postulate MoA

The following studies to test endocrine activity were performed, according to the EFSA/ECHA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009:

E modality: ToxCast Information as well as the Uterotrophic assay (OECD 440) (procedure to test for antioestrogenicity was not performed).

A modality: Hershberger bioassay in rats (OECD 441).

S modality: H295R steroidogenesis assay OECD 456 and the aromatase assay (human recombinant) OPPTS 890.1200.

All *in vitro* assays showed any kind of alteration induced by OPP. In addition, in Hershberger assay (B.6.8.3-07), accessory organ sex tissues were decreased, despite only ventral prostate was statistically significantly reduced. This may be related to the findings observed in the peripubertal male rat assay (B.6.8.3-09), where decreases in the weight of seminal vesicles plus coagulating glands with fluid, seminal vesicles plus coagulating glands without fluid, ventral prostate, LABC, left testis and epididymis, were observed (at a dose above MTD). Regarding females, in the pubertal assay (B.6.8.3-08), irregular oestrus cycle was noted (in studies ID 14 and 15 no differences in oestrous cycle were obseerved), which may be related to the inhibition of the aromatase seen *in vitro* (B.6.8.3-04).

However, as all 'EATS-mediated' parameters have not been investigated, additional information is requested (point 3.4.4.2 of the EFSA/ECHA guideline includes this possibility). In the scenario 2 (a)(i) where endocrine activity has been observed and where 'sensitive to, but not diagnostic of 'EATS' parameters' are observed and where the pattern of effects is deemed adverse, the biological plausibility that the adverse effects are (exclusively) caused via an endocrine-mediated MoA is not as strong as for the 'EATS-mediated' parameters. Nevertheless, these effects might provide indications of an endocrine MoA which warrant further investigation; in these cases, it is likely that further empirical data will need to be generated, e.g. levels 3, 4 and/or 5 on the substance under evaluation to demonstrate the link between the observed adverse effect and an endocrine MoA.

In studies ID 14 and 15 (OECD guideline 416) the following parameters were not assessed: age at balanopreputial separation, age at vaginal opening, anogenital distance, coagulating gland weight, epididymis weight, seminal vesicles weight, sperm morphology, sperm motility, sperm numbers, and uterus weight (with cervix). According to the EFSA document 'Outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology[34]', approved on March 2020, anogenital distance of each F1 and F2 pups, presence and number of nipples/areolae in all male F1 and F2 pups, histopathological assessment of the mammary gland in P0 and F1 adult males and females, and sperm parameters should be measured and reported as best scientific practice. Besides, there is a lack of fully reliable information from developmental studies (ID 16-20) (including fertility, pre and post-implantation loss, and litter/pup weight) and the main observed alterations in the peripubertal female and male rats assays (ID 31 and 32) were seen at a dose above MTD.

On the other hand, interaction of chemicals with the hypothalamic–pituitary–adrenal axis may affect both the developing immune and nervous systems. Sex hormones play an important role in development of sexual dimorphism of the brain; therefore, substances interfering with the sex hormonal signalling may affect the developing of this organ. In the studies submitted by the applicant, some kind of alterations were seen in brain. In rat, in studies ID 4 (13 weeks) and ID 10 (2 year); as well as in mouse ID 12 (2 year) at higher doses than 402 mg/kg/day. The only study in which this parameter was measured and not altered was in the 3-weeks dermal study in rat (ID 7) (these changes may be related to decreases in body weight). Pituitary weight, which was only analysed in 3 studies, presented alterations in two of them. In study ID 5 no alterations were observed (dogs were dosed only given 5 days per week and emesis was observed at all doses); but in studies ID 31 and 32, changes were seen. In study 31 there was a decrease in relative weight (9.8%); and in study 32 absolute (15%) and adjusted

34 Outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology European Food Safety Authority (EFSA). Approved 26 Mach 2020

(11%) weight were also decreased. In study ID 32 a male of the 900 mg/kg/day group presented pale pituitary (at these studies MTD was surpassed).

These facts together make it highly recommendable to perform a Two-Generation Reproduction Toxicity Study (OECD 416) or an Extended One-Generation Reproductive Toxicity Study (OECD 443). Due to the alterations observed in brain weight even at non-toxic doses (as seen in study ID 12, at the dose of 500 mg/kg/day), the alterations seen in foetuses (ID16, 17 and 20), and the observed antagonism of the androgen receptor (ID 25), which may lead to nipple retention, OECD 443 study may be preferred.

It should be noted that performing antiestrogenicity procedure of uterotrophic assay may support an impaired fertility in females MoA, but not dismiss it; and adversity parameters would be still pending to measure. On the other hand, repeating peripubertal female and male assays with appropriate doses, could confirm the results obtained at the dose above MTD, but level 5 information may be still needed. In the case the results were negative, more information on key parameters would be also still needed (alteration in testis weight and histopathology were observed in some studies. In addition, sperm parameters have not been addressed).

2.2.5 Conclusion of the assessment of EAS-modalities

Based on scenario 2a (i), it is considered that endocrine activity has been observed for the EAS-modalities. In addition, as explained above, there is only an outdated OECD TG 416 study available (and lack of OECD TG 443); therefore, based on the ED Guidance, the EAS-mediated parameters are considered not sufficiently investigated. In addition, the reliability of the results of reproductive toxicity (ID 14 and 15) and developmental toxicity studies (ID 16-20) is questionable. **Consequently, due to the lack of key parameters that were not measured or lack of reliability, it is considered that to draw a MoA more information is needed. Therefore, a Two-Generation Reproduction Toxicity Study (OECD 416) or an Extended One-Generation Reproductive Toxicity Study (OECD 443) should be conducted**.

2.3 Overall conclusion on the ED assessment for humans

The T-modality has been considered sufficiently investigated, T-mediated adversity and T-mediated endocrine activity have not been observed, corresponding to a scenario 1a. Therefore, it is considered that the ED criteria for T-modality are not met for OPP. The MoA analysis for this modality is not required.

On the other hand, considering the available data, EAS-mediated activity has been observed, but EAS- mediated adversity has not been sufficiently investigated, corresponding to a scenario 2a (i). In this particular case, as it was previously exposed, further data need to be generated to perform a MoA. It is considered that more information from a level 5 study is needed. Specifically, a Two-Generation Reproduction Toxicity Study (OECD 416) or an Extended One-Generation Reproductive Toxicity Study (OECD 443) should be conducted (the latter would be preferred).

It should be noted that no information on the sodium salt of 2-phenylphenol (NaOPP) was included. Therefore, an evaluation of its endocrine disrupting properties was not addressed.

3. Overall conclusion on the ED assessment

In conclusion, according to the current data, it is not considered that OPP be an endocrine disruptor for thyroid (scenario 1a). However, considering the available data, more information needs to be generated to reach a conclusion on EAS modalities (a scenario 2ai is proposed) in which endocrine activity has been observed but it is considered that level 5 studies are required to draw a MoA.

It should be noted that no information on the sodium salt of 2-phenylphenol (NaOPP) was included. Therefore, an evaluation of its endocrine disrupting properties was not addressed.

2.10.2 ED assessment for non-mammalian NTOs.

2.10.3 ED assessment for T-modality

Have T-mediated parameters been sufficiently investigated?

Yes, based on a conclusive test according to OCED 231 (Amphibian metamorphosis assay (AMA).

2.10.4 Lines of evidence for adverse effects and endocrine activity related to T-modality

Table 2.10.2-1: Assembled lines of evidence for non-target organisms – T-modality

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
34	EATS-mediated	Thyroid histopathology (amphibian)	Xenopus laevis	21 d	Uptake from water	>1.92	mg/L water	No effect	No effect	Conclusive, unspecific developmental delay at high dose, no histological change of thyroid	Conclusive evidence for absence of T-related adverse effects	T
34	EATS-mediated	Hind limb length	Xenopus laevis	21 d	Uptake from water	>1.92	mg/L water	No effect	No effect			
34	EATS-mediated	Developmental stage	Xenopus laevis	21 d	Uptake from water	>1.92	mg/L water	Change	Slight delay at Day 21, no effect on Day 7			
34	Sensitive to, but not diagnostic of, EATS	Snout-vent length/growth	Xenopus laevis	21 d	Uptake from water	>1.92	mg/L water	No effect	No effect	Conclusive		

2.10.5 Assessment of the integrated lines of evidence and weight

The following assessment was provided by the applicant:

- *WoE for T-mediated adversity*

The amphibian metamorphosis assay (AMA) did not show a specific adverse effect. The slight delay of development at the highest test concentration is not regarded as a specific T-mediated effect since thyroid histology and hind-limb length were not affected.

- *WoE for T-mediated endocrine activity*

As stated above, there were no treatment-related effects in the AMA.

RMS conclusion

To consider the T-modality sufficiently investigated, an ‘Amphibian metamorphosis assay’ (AMA;OECD TG 231 (OECD, 2009c)) should be conducted. The following AMA study is available:

- Lehman C., Hutchinson K., Fiting J., Thomas J., 2012. Guideline OPPTS 890.1100 ; OECD 231

The study are considered valid. The following parameters were investigated: Hind limb length, Thyroid histological, Snout–vent length, Wet weight and Developmental stages.
There were no indications of developmental delay or advanced development (as measured by developmental stage and hind limb length), nor were there any signs of asynchronous development among OPP-exposed tadpoles relative to control tadpoles on day 7.

Tadpoles exposed to 1.92 mg/L OPP demonstrated delayed development compared to controls on day 21. According to the guideline, delayed development is not by itself an indicator of anti-thyroidal activity and needs to be confirmed by histopathological analysis of the thyroid. In this case, there were no treatment-related histopathological effects observed in the thyroid glands from OPP-exposed tadpole compared with controls. This could be indicative of some generalized toxicity to these tadpoles at the highest concentration of OPP tested.

The overall WoE suggests that T-mediated parameters have been sufficiently investigated and T-mediated adversity was not observed across the different studies conducted, at different doses, species, and lengths of treatment. Therefore, the ED criteria are not met for this modality according to a scenario 1a.

2.10.5.1.1 Initial analysis of the evidence and identification of the relevant scenario

Table 2.10.2.1.3-1: Selection of relevant scenario

Adversity based on T-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected** ***(indicate with an "x" the scenario selected based on the assessed lines of evidence)***
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "T-mediated" adversity	x
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no T-mediated endocrine activity observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.10.5.1.2 Conclusion on the ED assessment for T-modality

No other endpoints were statistically significant nor were there signs of asynchronous or advanced development. **Therefore, OPP is considered "likely thyroid inactive" in the Amphibian Metamorphosis Assay.** Since the T-mediated parameters has been sufficiently investigated, it corresponds with a scenario 1a.

2.10.5.2 ED assessment for EAS-modality

Have EAS-mediated parameters been sufficiently investigated?

Yes, based on a conclusive test according to OCED OECD 229 (Fish short term reproduction assay).

2.10.5.2.1 Lines of evidence for adverse effects and endocrine activity related to EAS-modalities

The following lines of evidence tables for EAS-mediated adversity and activity are available:

Table 2.10.2.2.1-1: Assembled lines of evidence for non-target organisms – EAS-modality

Study ID Matrix	Grouping	Lines of evidence	Species	Duration of exposure	Route of administration	Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
33	In vivo mechanistic	Vitellogenin (VTG) in females	fathead minnow	21 d	Uptake from water	>0.876	mg/L water	No effect	No effect	Conclusive	Conclusive in-vivo mechanistic evidence for absence of E-related activity	EAS
33	In vivo mechanistic	Vitellogenin (VTG) in males	fathead minnow	21 d	Uptake from water	>0.876	mg/L water	No effect	No effect	Conclusive		EAS
33	EATS-mediated	Male 2nd sex characteristics in males	fathead minnow	21 d	Uptake from water	>0.876	mg/L water	No effect	No effect	Conclusive	Conclusive in-vivo evidence for absence of E-related effects on apical endpoints	EAS
33	EATS-mediated	Specific gonad histopathology	fathead minnow	21 d	Uptake from water	>0.876	mg/L water	No effect	No effect	Conclusive		EAS
33	Sensitive to, but not diagnostic of, EATS	Gonado-somatic index	fathead minnow	21 d	Uptake from water	>0.876	mg/L water	No effect	No effect	Conclusive		EAS
33	Sensitive to, but not diagnostic of, EATS	Reproduction (fecundity, fertility)	fathead minnow	21 d	Uptake from water	>0.876	mg/L water	Decrease	Decreased fecundity & fertility at toxic concentrations	Inconclusive		EAS

2.10.5.2.2 Assessment of the integrated lines of evidence and weight

The following assessment was provided by the applicant:

- *WoE for EAS-mediated adversity*

In the fish short-term reproductive toxicity assay, reduced fecundity and fertility was observed at the highest test concentration, which caused 29% mortality. This effect can therefore not be regarded as a specific endocrine effect.

- *WoE for EAS-mediated endocrine activity*

There is conclusive in-vivo mechanistic evidence for absence of EAS-related activity.

RMS conclusion
According to the Guidance for the identification of endocrine disruptors, to consider the E, A, S modalities for non-target organisms other than mammals sufficiently investigated, preferably the 'Fish short term reproduction assay' (FSTRA; OECD TG 229) should have been conducted; however the 21-day fish assay OECD TG 230 (OECD, 2009b) is acceptable as well.
There are two fish short-term reproduction assay conducted with fish availables:

- Caunter J., Williams T., 2002 (KCA 8.2.2.1/01). Guidelines: 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
- Lehman C., et al., 2012, revised 2015 (KCA 8.2.3/01). Guidelines: OPPTS 890.1350 ; OECD 229

The following parameters were investigated: Mortality, behaviour and Apperance, Fecundity and Fertility, Weigth and Length, Gonado-Somatic Index (GSI), Vitellogenin and Gonad histopatology. There were no significant treatment related effects on specific endocrine-responsive endpoints, such as vitellogenin concentrations, gonado somatic indices or tubercle scores. Therefore, **the results indicated that OPP does not have potential for endocrine activity in the HGP axis of the fish**. Since the EATS-mediated parameters have been sufficiently investigated, it corresponds with a scenario 1a.

2.10.5.2.3 Initial analysis of the evidence and identification of the relevant scenario

Table 2.10.2.2.2-1: Selection of relevant scenario

Adversity based on T-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected** ***(indicate with an "x" the scenario selected based on the assessed lines of evidence)***
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "EAS-mediated" adversity	x
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no T-mediated endocrine activity observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.10.5.2.4 Conclusion on the ED assessment for EAS-modality
EAS--mediated parameters were sufficiently investigated, Scenario 1a is applied and the ED criteria are not met for this modality for non-target organism other than mammals.

2.10.6 Overall conclusion on the ED assessment

In conclusion, according to the current data, it is not considered that OPP be an endocrine disruptor for thyroid (scenario 1a). However, considering the ED assessment for human health, more information needs to be generated to reach a conclusion on EAS modalities (a scenario 2ai is proposed) in which endocrine activity has been observed but it is considered that level 5 studies are required to draw a MoA.

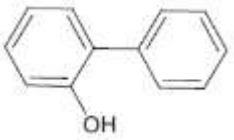
It should be noted that no information on the sodium salt of 2-phenylphenol (NaOPP) was included. Therefore, an evaluation of its endocrine disrupting properties was not addressed.

2.11 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA *[SECTIONS 1-6 OF THE CLH REPORT]*

2.11.1 Identity of the substance [section 1 of the CLH report]

2.11.1.1 Name and other identifiers of the substance

Table 69: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2-phenylphenol, *o*-phenylphenol (IUPAC) [1,1'-Biphenyl]-2-ol (CA)
Other names (usual name, trade name, abbreviation)	OPP
ISO common name (if available and appropriate)	2-phenylphenol (ISO)
EC number (if available and appropriate)	201-993-5
EC name (if available and appropriate)	biphenyl-2-ol
CAS number (if available)	90-43-7
Other identity code (if available)	246
Molecular formula	$C_{12}H_{10}O$
Structural formula	
SMILES notation (if available)	Oc2ccccc2c1ccccc1
Molecular weight or molecular weight range	170.2 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	The active substance is not a mixture of isomers. Therefore, consideration of isomeric composition is not relevant.
Description of the manufacturing process and identity of the source (for UVCB substances only)	CONFIDENTIAL information - data provided separately (Volume 4)
Degree of purity (%) (if relevant for the entry in Annex VI)	998 g/kg minimum

2.11.1.2 Composition of the substance

Table 70: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
2-phenylphenol, *o*-phenylphenol (OPP)	99.8 % minimum	Skin Irrit. 2- H315 Eye Irrit. 2 - H319 STOT SE 3- H335 Aquatic Acute 1- H400	GHS09 GHS07 Wng

Table 71: Impurities (non-confidential information) if relevant for the classification of the substance

2-phenylphenol does not contain relevant impurities.

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**

Table 72: Additives (non-confidential information) if relevant for the classification of the substance

2-phenylphenol does not contain additives.

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**

Table 73: Test substances (non-confidential information)

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**	**The study(ies) in which the test substance is used**
2-phenylphenol, *o*-phenylphenol (OPP)	99.8 % minimum	none	N/A	Melting point/Boiling point
				Vapour pressure
				Solubility in water
				Partition coefficient octanol/water
				Dissociation constant
				Flammability/self-heating
				Flash point
				Explosive properties
				Oxidising properties

2.11.2 Proposed harmonized classification and labelling

2.11.2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 74: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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 GSH09 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	**Add** Carc. 2 Aquatic Chronic 1 **Modify** Skin Corr. 1 Eye Dam. 1 **Remove** STOT SE 3 **Retain** Aquatic Acute 1	**Add** H351 H410 **Modify** H314 H318 **Remove** H335 **Retain** H400	**Add** GHS08 GHS05 **Remove** GHS07 **Modify** Dgr **Retain** GSH09	**Add** H351 H410 **Modify** H314 H318 **Remove** H335 **Retain** H400		Add M = 1 M = 1	
Resulting Annex VI entry if agreed by RAC and 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 Aquatic Acute 1 Aquatic Chronic 1	H351 H314 H318 H400 H410	GHS05 GHS08 GSH09 Dgr	H351 H314 H410		Add M = 1 M = 1	

2.11.2.2 Additional hazard statements / labelling

Table 75: Reason for not proposing harmonised classification and status under CLH public consultation

Hazard class	Reason for no classification	Within the scope of CLH consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	-
Oxidising gases	Hazard class not applicable	-
Gases under pressure	Hazard class not applicable	-
Flammable liquids	Hazard class not applicable	-
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances	Data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Hazard class not applicable	-
Pyrophoric solids	Data conclusive but not sufficient for classification	Yes
Self-heating substances	Data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	-
Oxidising liquids	Hazard class not applicable	-
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Hazard class not applicable	-
Corrosive to metals	Data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	**Harmonised classification proposed**	Yes
Serious eye damage/eye irritation	**Harmonised classification proposed**	Yes
Respiratory sensitisation	Data lacking	Yes
Skin sensitisation	Data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	Data inconclusive	Yes
Carcinogenicity	**Harmonised classification proposed**	Yes
Reproductive toxicity	Data conclusive but not sufficient for classification.	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Data conclusive but not sufficient for classification	Yes
Hazardous to the aquatic environment	Harmonised classification proposed	Yes

Hazard class	Reason for no classification	Within the scope of CLH consultation
Hazardous to the ozone layer	Data lacking	Yes

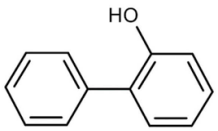
2.11.3 History of the previous classification and labelling

2.11.4 Identified uses

2.11.5 Data sources

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.

2.12 RELEVANCE OF METABOLITES IN GROUNDWATER

No significant metabolites were detected in the aerobic soil metabolism study of OPP, therefore, there are no metabolites of concern for groundwater

2.12.1 Overall conclusion

No significant metabolites were detected in the aerobic soil metabolism study of OPP, therefore, there are no metabolites of concern for groundwater

2.13 CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

The active substance is not a mixture of isomers, therefore consideration of isomeric composition is not relevant.

2.13.1 Identity and physical chemical properties

The active substance is not a mixture of isomers, therefore consideration of isomeric composition is not relevant.

2.13.2 Methods of analysis

The active substance is not a mixture of isomers, therefore consideration of isomeric composition is not relevant.

2.13.3 Mammalian toxicity

The active substance is not a mixture of isomers, therefore consideration of isomeric composition is not relevant.

2.13.4 Operator, Worker, Bystander and Resident exposure

The active substance is not a mixture of isomers, therefore consideration of isomeric composition is not relevant.

2.13.5 Residues and Consumer risk assessment

The active substance is not a mixture of isomers, therefore consideration of isomeric composition is not relevant.

2.13.6 Environmental fate

The active substance is not a mixture of isomers, therefore consideration of isomeric composition is not relevant.

2.13.7 Ecotoxicology

The active substance is not a mixture of isomers, therefore consideration of isomeric composition is not relevant.

2.14 RESIDUE DEFINITIONS

2.14.1 Definition of residues for exposure/risk assessment

Food of plant origin: Sum of 2-phenylphenol and phenylhydroquinone and their salts and conjugates, expressed as 2-phenylphenol (only for fruit crops).

Food of animal origin: 2-phenylphenol (by default)

Soil: 2-phenylphenol

Groundwater: 2-phenylphenol

Surface water: 2-phenylphenol, Diketohydroxy-compound ((2-hydroxy-1,2-dihydrodibenzo[b,d]furan3,4-dione))

Sediment: 2-phenylphenol

Air: 2-phenylphenol

2.14.2 Definition of residues for monitoring

Food of plant origin: 2-phenylphenol (sum of 2-phenylphenol and its conjugates, expressed as 2-phenylphenol), (only for fruit crops).

Food of animal origin: 2-phenylphenol (by default).

Soil: 2-phenylphenol

Groundwater: 2-phenylphenol

Surface water: 2-phenylphenol, 2-phenylphenol

Sediment: 2-phenylphenol

Air: 2-phenylphenol

Body fluids and tissues (toxicology): (2-phenylphenol and 2-phenylphenol sodium salt), its sulphate and glucuronide conjugates (major phase II metabolites).

Level 3

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

3 PROPOSED DECISION WITH RESPECT TO THE APPLICATION

3.1 BACKGROUND TO THE PROPOSED DECISION

3.1.1 Proposal on acceptability against the decision making criteria – Article 4 and annex II of regulation (EC) No 1107/2009

3.1.1.1 Article 4

		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	X		**Efficacy:** OPP is used as a post-harvest treatment for control of fungi in citrus fruits. The key pests include, but are not restricted to: • *Penicillium digitatum* • *Penicillium italicum* • *Phomopsis citri* **Operator, bystander/resident and worker:** The operator, bystander/resident and worker risk assessment demonstrates acceptable risk to 2-phenylphenol for the proposed use of AGF/1-04 for operators and workers. However, AGF/1-04 with regards to human health is classified as Carc. 2 (H351), and based on this classification and the requirement for chemical protective gloves for workers, the following PPE are recommended: • Operator: Work wear (arms, body and legs covered) and chemical protective gloves when handling the concentrate, or handling contaminated surfaces. NOTE: according EFSA Guidance, 2014, the penetration factor of the "workwear" is 10 %, equivalent to a type 6 chemical protective coverall (or the correspondent coverall according UNE-EN ISO 27065:2017) • Worker: Work wear (arms, body and legs covered) and chemical protective gloves when handling treated fruits. **Consumer risk assessment:** The highest exposure is for the DE child at 16% of the ADI, with oranges contributing 13%. The long-term estimated dietary intake is therefore below the ADI and a risk to the consumer is unlikely.

				Environmental fate and behavior and Ecotoxicology section AGF/1-04, is an EC formulation containing 100 g/L OPP. The product is applied indoors, in packing houses. Harvested citrus fruits already packed in boxes are passed through a closed system where they are showered with product diluted in water. The used diluted product as well as the cleaning waters are treated as chemical waste in accordance with local legislation. There is no exposure to the environment. Nevertheless, to simulate potential contamination of surface waters via emission from sewage treatment plants (STP), PEC_{sw} and PEC_{sed} values have been calculated from $PEC_{effluent}$ values, which were modelled using SimpleTreat version 3.1 and SimpleTreat version 4.0. These PECsw calculations and the aquatic risk assessment provided have been considered as additional information. PEC_{soil} and PECgw values have not been calculated. **Ecotoxicology:** The ecotoxicological risk assessment demonstrates acceptable risk to 2-phenylphenol for the proposed use of AGF/1-04 for non target species.

3.1.1.2 Submission of further information

		Yes	No	
i)	It is considered that a complete dossier has been submitted	X		A complete dossier was submitted according to data requirements. However, during evaluation some data gaps were identified.
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.	X		The manufacturing sites of OPP technical shall be identified as well as the original technical grade active substance TC/TK used to manufacture the formulated product. Signed LoS for each formulation manufacturing sites are required according to the information provided in Vol.4, confidential. The formulation composition and the origin of the SOPP batches from OPP shall be corroborated with a composition certificate. SDS for OPP technical shall be provided. SDS for CAS 57-55-6 should be given in English as well as SDS for AGF1-04 formulation. Full detailed description of the intended commercial containers for the plant protection product shall be provided in line with the storage stability results

				within Vol.3, CP, B.2.
3.1.1.3 Restrictions on approval				
		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.	X		OPP is used as a post-harvest treatment
3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		**OPP:** ADI = 0.40 mg/kg bw/day ARfD : has not been deemed required AOEL= 0.40 mg/kg bw/day AAOEL: has not been deemed required **SOPP:** No ADI can be allocated due to lack of data. No ARfD value can be established due to lack of data. No AOEL can be allocated due to lack of data. No AAOEL can not be allocated due to lack of data. **PHQ:** The amount of PHQ metabolite formed in rats is less than 10 % and therefore, the migration of the reference value from the parent may not be applied. No reference value can be determined for PHQ so the toxicological relevance of this metabolite remains to be determined: • Gene mutation: This may be covered by the QSAR analysis • Aneugenicity/Clastogenicity: Data gap • Repeat dose (extended 28-day or 90-day studies): Data gap
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In	X		The highest exposure is for the DE child at 16% of the ADI, with oranges contributing 13%. The long-term estimated dietary intake is therefore below the ADI and a risk to the consumer is unlikely.

	particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.			
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		AGF/1-04, is an EC formulation containing 100 g/L OPP. The product is applied indoors, in packing houses. Harvested citrus fruits already packed in boxes are passed through a closed system where they are showered with product diluted in water. The used diluted product as well as the cleaning waters are treated as chemical waste in accordance with local legislation. There is no exposure to the environment. Nevertheless, to simulate potential contamination of surface waters via emission from sewage treatment plants (STP), PEC_{sw} and PEC_{sed} values have been calculated from $PEC_{effluent}$ values, which were modelled using SimpleTreat version 3.1 and SimpleTreat version 4.0. These PECsw calculations and the aquatic risk assessment provided have been considered as additional information. PEC_{soil} and PECgw values have not been calculated.
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		OPP is used as a post-harvest treatment for control of fungi in citrus fruits. The key pests include, but are not restricted to *Penicillium digitatum*, *Penicillium italicum*, *Phomopsis citri*. OPP shows multi-site activity in fungi. It is adsorbed to the fungal cell membrane, where it disturbs cell membrane functions, such as substrate transport and ATP synthesis. The cell membrane loses its semi-permeability leading to loss of organic molecules and ions. The representative formulation, AGF/1-04, is currently commercially available and supported by efficacy data evaluated under Uniform Principles

				for national registration. OPP is not specifically listed in the Fungicide Resistance Action Committee FRAC Code List of 2018. There is no know OPP resistance in the EU of fungal species causing storage spoilage of citrus fruits. Adverse effects are not likely to occur in treated crops as the application is a post-harvest treatment on harvested citrus fruits. There is no exposure to citrus trees. There are no other undesirable or unintended side effects resulting from the use of OPP according to good agricultural practice. There is no exposure to growing crops or non-target organisms.
Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.	X		No reference value can be determined for PHQ so the toxicological relevance of this metabolite remains to be determined: • Gene mutation: This may be covered by the QSAR analysis • Aneugenicity/Clastogenicity: Data gap • Repeat dose (extended 28-day or 90-day studies): Data gap **Environmental relevance of metabolites:** there are no relevant metabolites in soil/groundwater.
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.	X		2-phenylphenol does not have relevant impurities or additives. None of the impurities present in the active ingredient is of ecotoxicological or environmental concern. Since OPP (Preventol O) is used to ´formulate´ SOPP (Preventol ON) the origin of the SOPP batches shall be demonstrated through a certificate including the details of the original OPP batches used. Otherwise the technical specifications for OPP would not be comparable to the intended SOPP used as technical grade active ingredient. **Refer to Volume 4, confidential for more details on composition.**
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.			No FAO specification is available at the time of submission.
	It is considered for reasons of protection of human or animal health or			**Not applicable. No FAO specification is available.**

	the environment, stricter specifications than that provided for by the FAO specification should be adopted			
Methods of analysis				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.	X		**Refer to Volume 4, confidential for more details on composition.** None of the impurities present in the active ingredient is of ecotoxicological or environmental concern.
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.		X	<u>Method of analysis for body fluids/tissues:</u> The extraction procedure shall guarantee its efficiency in the analysis of the sulphate and glucuronide conjugates (major phase II metabolites) included in the residue definition for body fluids and tissues (toxicology): *The active substance (OPP and SOPP) and its sulphate and glucuronide conjugates (major phase II metabolites).* PHQ metabolite is excluded from the residue definition. Method and validation of method *Bacher, R., Heinz, N. (2019)* [KCA 4.2/4] seems suitable for OPP analysis, however, there is no previous hydrolysis step and it is unclear, to which extent, all the components in the residue definition can be determined for body fluids/ tissues (toxicology).
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		
Impact on human health				
Impact on human health - ADI, AOEL, ARfD				
		Yes	No	•
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		The following reference values for *ortho*-phenylphenol and sodium *ortho*-phenylphenate are: **<u>OPP:</u>** ADI = 0.40 mg/kg bw/day ARfD : has not been deemed required AOEL= 0.40 mg/kg bw/day

				AAOEL: has not been deemed required **SOPP:** No ADI can be allocated due to lack of data. No ARfD value can be established due to lack of data. No AOEL can be allocated due to lack of data. No AAOEL can not be allocated due to lack of data. **PHQ:** The amount of PHQ metabolite formed in rats is less than 10 % and therefore, the migration of the reference value from the parent may not be applied. No reference value can be determined for PHQ so the toxicological relevance of this metabolite remains to be determined: • Gene mutation: This may be covered by the QSAR analysis • Aneugenicity/Clastogenicity: Data gap • Repeat dose (extended 28-day or 90-day studies): Data gap
Impact on human health – proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**		X	No human data are available for OPP or SOPP, hence classification as Category 1 is not possible. All available *in vivo* germ and somatic cells mutagenicity assay data with OPP do not meet the criteria for classification. However, based on the low reliability of these data and on the undetermined evaluation of clastogenicity *in vivo*, the conclusion for no classification and labelling cannot be drawn for OPP. All available *in vivo* somatic cell mutagenicity assay data with SOPP do not meet the criteria for classification. However, based on the low reliability of these data, the conclusion for no classification and labelling cannot be drawn for SOPP.
Impact on human health – proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by		X	In long-term/carcinogenicity studies, urinary bladder hyperplasia and transitional cell carcinoma were observed in male rats starting at doses of 8000 ppm (approximately 400 mg/kg bw), while increased incidence of hepatocellular adenoma in mice was observed at doses of 500 mg/kg and higher.

	the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**			The results of the new mechanistic studies indicate that: PPARα activation is the most likely MoA for the liver adenomas in mice (this MoA is generally recognised as not relevant for humans as explicitly mentioned in CLP). In addition, the liver tumours where only found in mice and in a strain in which they are particularly frequent (Maronpot *et al.*, 1987). So these hepatocellular tumours should be assigned little weight in the assessment of the carcinogenic potential of OPP. The MoA for bladder tumour formation in male rats is likely to be non-genotoxic (involving urothelium irritation and dependant on pH and sodium concentration), and in a species known to be more susceptible to bladder tumours as a response to chronic irritation than humans (Rodent Bladder Carcinogenesis Working Group, 1995). However, the MoA that led to the formation of these tumours remains unknown, thus the proposed classification is carcinogen category 2 (H351).
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			*[if no provide a brief explanation of conditions of use and cross refer to the section containing full details to support the contention of negligible exposure]*
Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**		X	The reproductive toxicity of OPP has been adequately investigated in rat multigenerational studies and in rat and rabbit developmental toxicity studies. These studies demonstrated that OPP does not possess hazardous properties in relation to fertility, reproductive performance or development. Classification for reproductive toxicity is not warranted.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in			*[if yes provide a brief explanation of conditions of use and cross refer to the section containing full details to support the contention of negligible exposure]*

	closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Impact on human health – proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE identified as having endocrine disrupting properties** in accordance with the provisions of point 3.6.5 in Annex II of Regulation (EC) No 1107/2009		?	**It is considered that endocrine disruption of the EAS modalities cannot be discarded.** Several *in vitro* studies (ER binding, AR binding, Steroidogenesis assay and Aromatase assay) suggest that there is endocrine activity. In the *in vivo* Hershberger assay, a statistically significant alteration in ventral prostate is observed, which could be related to the observed effects in ASO and tissues (*i.e.* ventral prostate, seminal vesicle, glans penis, Cowper's glands) in the thyroid pubertal male assay at a dose above MTD (however, the second highest dose is too low according to the corresponding guideline to dismiss potential effects). A delay in balanopreputial separation was also observed. In this same dose-selection condition, in thyroid peripubertal females assay, alterations in oestrous cycle regularity were observed at the highest dose. On the other hand, key parameters in the long-term and prenatal developmental studies have not been measured. Consequently, it is considered that more information would be necessary to perform a MoA on EAS modalities. According to the EFSA/ECHA guidance, a level 5 study (OECD 443/416) may be considered. Regarding thyroid, it is considered that no adversity has been observed. In the absence of SOPP studies, information to establish a relationship between OPP studies and its salt, or their equivalence, evaluation of OPP sodium salt have not been conducted.
ii)	Linked to above identification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in			*[if yes provide a brief explanation of conditions of use and cross refer to the section containing full details to support the contention of negligible exposure]*

	accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	***1.- <u>Persistence criterion</u>*** ***Soil system****: The aerobic degradation of OPP was studied in a sandy loam soil soil under laboratory conditions. Trigger (persistence) DT_{50} and DT_{90} values at 20 ºC and 50% MWHC were calculated to be 2.4 and 11.1 hours respectively.* *The anaerobic degradation of OPP was not considered.* *The photodegradation of OPP was investigated on a sandy clay loam soil under aerobic conditions. The single first order half-life of OPP was 0.13 days, (light) and 0.16 days (dark).* *Photodegradation contributes slightly to increase the degradation rate with the formation of three unknown metabolites (the sum of % AR associated with these metabolites accounted for less than 7.5%)* *<u>Overall, 2-Phenylphenol does not fulfill the persistence criterion in soil set out in points 3.7.1.1 (POP criteria), 3.7.2.1 (PBT criteria), 3.7.3.1 (vPvB criteria) of annex II of the regulation 1107/2009.</u>* ***Aquatic system:*** *OPP was determined to be hydrolytically stable, degrading by less than 10% after 5 days at 50°C in pH4, pH7 and pH9 buffers.* *OPP degraded rapidly in the aqueous phototransformation test. The DT50 of OPP was 0.3 days, equivalent to 1.7 solar summer days in Phoenix, Arizona (33.3°N) or 2.6 summer days in Athens, Greece (38.0°N).* *In another laboratory study the direct and the direct plus indirect aqueous photolysis of 2-phenylphenol was investigated in pure water and in contaminated natural lake water using natural sunlight. The direct photodegradation rate of 2-phenylphenol observed in pure water under summer sunlight was 0.13 d^{-1} (DT50 = 5.3 days). In lake water, the direct-plus-indirect photolysis rate constant was of*

				0.15d $^{-1}$. *OPP was determined to be readily biodegradable in the OECD 301B CO_2 evolution test, the OECD 301E modified OECD screening test and the OECD 302B Zahn-Wellens test.* *The biotic degradation of OPP was elucidated based on the results of studies to determine the toxicity of OPP to chironomids. The generated data were only intended for screening purposes. In all tests the amount of OPP detectable via chemical analysis was reduced by 50% or more within 14 days (DT50 <14 d).* *The aerobic mineralization was not investigated.* *Overall, 2-Phenylphenol does not fulfil the persistence criterion in aquatic systems set out in points 3.7.1.1 (POP criteria), 3.7.2.1 (PBT criteria) and 3.7.3.1 (vPvB criteria) of annex II of the regulation 1107/2009.* ***2.- Bioaccumulation criterion*** *The log Pow of 2-phenylphenolis > 3. Available laboratory study on aquatic organisms show BCF below 2000.* ***3.-.Toxicity criterion*** *Available studies on aquatic organisms show No-observed effect concentration below 0.01 mg/l.* ***4.- Atmospheric long range transport*** *The atmospheric half-life of the active substance 2-Phenylphenol was estimated to be 0.59 days, bellow the trigger value of 2 d. Based on these calculations, 2-Phenylphenol is not expected to be subjected to long-range transport in the atmosphere.*
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	See previous paragraph
Very persistent and very bioaccumulative substance (vPvB).				

		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	See previous paragraph
Ecotoxicology				
		Yes	No	
i	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.	X		AGF/1-04, is an EC formulation containing 100 g/L OPP. The product is applied indoors, in packing houses. Harvested citrus fruits already packed in boxes are passed through a closed system where they are showered with product diluted in water. The used diluted product as well as the cleaning waters are treated as chemical waste in accordance with local legislation. There is no exposure to the environment.
ii	It is considered that, on the basis of the assessment of Community or internationally agreed test guidelines, the substance **SHOULD BE identified as having endocrine disrupting properties HAS** endocrine disrupting properties that may cause adverse effects on non-target organisms in accordance with the provisions of point 3.8.2 in Annex II of Regulation (EC) No 1107/2009.		X	Results of the two available fish short-term reproduction assay indicated that OPP does not have potential for endocrine activity in the HGP axis of the fish. Since the EATS-mediated parameters have been sufficiently investigated, it corresponds with a scenario 1a. Results of an 'Amphibian metamorphosis assay' (AMA;OECD TG 231 (OECD, 2009c)) showed no indications of developmental delay or advanced development (as measured by developmental stage and hind limb length), nor were there any signs of asynchronous development among OPP-exposed tadpoles relative to control tadpoles on day 7. OPP is considered "likely thyroid inactive" in the Amphibian Metamorphosis Assay. Since the T-mediated parameters has been sufficiently investigated, it corresponds with a scenario 1a.
iii	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.			*[Explain if this applies to all or some of the representative uses/use scenarios/products]*
iv	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or			*[Insert brief overall summary of honey bee assessments here. Cross refer to level 2 as necessary]* *[Explain if this applies to all or some of the representative uses/use scenarios/products]*

	synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.			
Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.	X		**Residue definitions for risk assessment** **Food of plant origin:** Sum of 2-phenylphenol and phenylhydroquinone and their salts and conjugates, expressed as 2-phenylphenol (only for fruit crops). **Food of animal origin:** 2-phenylphenol (by default) **Soil:** 2-phenylphenol **Groundwater:** 2-phenylphenol **Surface water:** 2-phenylphenol, Diketohydroxy-compound ((2-hydroxy-1,2-dihydrodibenzo[b,d]furan3,4-dione)) **Sediment:** 2-phenylphenol **Air:** 2-phenylphenol **Residue definitions for monitoring** **Food of plant origin:** 2-phenylphenol (sum of 2-phenylphenol and its conjugates, expressed as 2-phenylphenol), (only for fruit crops). **Food of animal origin:** 2-phenylphenol (by default). **Soil:** 2-phenylphenol **Groundwater:** 2-phenylphenol **Surface water:** 2-phenylphenol, 2-phenylphenol **Sediment:** 2-phenylphenol **Air:** 2-phenylphenol **Body fluids and tissues:** [2-phenylphenol and 2-phenylphenol sodium salt], its sulphate and glucuronide conjugates (major phase II metabolites)
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the	X		AGF/1-04, is an EC formulation containing 100 g/L OPP. The product is applied indoors, in packing houses. Harvested citrus fruits already packed in boxes are passed through a closed system where they are showered with product diluted in water. The used diluted product as well as the cleaning waters are treated as chemical waste in accordance with local legislation.

	respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.			There is no exposure to the environment

3.1.2 Proposal – Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution		X	

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** If the active substance is not a micro-organism, in particular it is considered that: (a) the substance **should NOT be classified or proposed for classification** in accordance to Regulation (EC) No 1272/2008 as any of the following: — carcinogenic category 1A, 1B or 2, — mutagenic category 1A, 1B or 2, — toxic to reproduction category 1A, 1B or 2, — skin sensitiser category 1, — serious damage to eye category 1, — respiratory sensitiser category 1, — acute toxicity category 1, 2 or 3, — specific Target Organ Toxicant, category 1 or 2, — toxic to aquatic life of acute and chronic category 1 on the basis of appropriate standard tests, — explosive, — skin corrosive, category 1A, 1B or 1C; (b) it has **not been identified as priority substance under Directive 2000/60/EC**; (c) it is **not deemed to be an endocrine disruptor** in accordance to Annex II of Regulation (EC) No 1107/2009; (d) it **has no neurotoxic or immunotoxic effects**; (e) it **is not persistent** (half-life in soil is more than 60 days) or its **bio-concentration factor is lower than 100**. (f) it is a **semiochemical** and verifies points (a) to (d).		X	The preliminary assessment of OPP includes the proposed classification as Carcinogenic, Category 2 (H351), hence OPP shall not be considered of low risk active substance.

	Paragraph (e) doesn't apply to naturally occurring active substances. If the active substance is a micro-organism, in particular it is considered that at strain level the micro-organism has not demonstrated multiple resistance to anti-microbials used in human or veterinary medicine. If the active substance is a baculovirus, in particular it has not demonstrated adverse effects on non-target insects.			

3.1.4 List of studies to be generated, still ongoing or available but not peer reviewed

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	**Study on-going and anticipated date of completion**	**Study available but not peer-reviewed**
3.1.4.1 Identity of the active substance or formulation				
None				
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
None				
3.1.4.3 Data on uses and efficacy				
None				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
None				
3.1.4.5 Methods of analysis				
None				

3.1.4.6 Toxicology and metabolism				
Based on the positive result in the *in vitro* chromosome aberration test, the potential clastogenicity *in vivo* has not been adequately addressed. The lack of clastogenicity *in vivo* must be demonstrated taking into consideration the formation of the metabolite PHQ, which is genotoxic.	All intended uses			
Based on the available Level 2 and 3 endocrine studies, which suggest endocrine activity, and on the lack of measured key Level 4 and 5 parameters, according to the EFSA/ECHA guidance, it is asked to conduct an in vivo study (specifically a Level 5 study OECD 443/416) to dismiss the possibility of potential endocrine adverse effects.	All intended uses			
Gene mutation for PHQ: This may be covered by the QSAR analysis	All intended uses			
Aneugenicity/Clastogenicity for PHQ	All intended uses			
Repeat dose (extended 28-day or 90-day studies) for PHQ	All intended uses			
3.1.4.7 Residue data				
None				

3.1.4.8 Environmental fate and behaviour				
None				
3.1.4.9 Ecotoxicology				
None				

3.1.5 Issues that could not be finalised

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

Area of the risk assessment that could not be finalised on the basis of the available data	**Relevance in relation to representative use(s)**
Toxicological relevance of the metabolite PHQ	All representative uses

3.1.6 Critical areas of concern

An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

Critical area of concern identified	**Relevance in relation to representative use(s)**
Toxicological relevance of the metabolite PHQ	All representative uses

3.1.7 Overview table of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)
All columns are grey as the material tested in the toxicological studies has not been demonstrated to be representative of the technical specification.

Representative use		Use "A" (X[1])	Use "B" (X[1])
Operator risk	Risk identified		
	Assessment not finalised		
Worker risk	Risk identified		
	Assessment not finalised		
Bystander risk	Risk identified		
	Assessment not finalised		
Consumer risk	Risk identified		
	Assessment not finalised		
Risk to wild non target terrestrial vertebrates	Risk identified		
	Assessment not finalised		
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified		
	Assessment not finalised		
Risk to aquatic organisms	Risk identified		
	Assessment not finalised		
Groundwater exposure active substance	Legal parametric value breached		
	Assessment not finalised		
Groundwater exposure metabolites	Legal parametric value breached		
	Parametric value of 10µg/L[(a)] breached		
	Assessment not finalised		
Comments/Remarks			

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	**Justification**
None	*[specify the reasons why expert consultation is considered necessary]*

3.1.9 Critical issues on which the Co RMS did not agree with the assessment by the RMS

Points on which the co-rapporteur Member State did not agree with the assessment by the rapporteur member state. Only the points relevant for the decision making process should be listed.

Issue on which Co-RMS disagrees with RMS	**Opinion of Co-RMS**	**Opinion of RMS**

3.2 PROPOSED DECISION

It is proposed that:

Approval of 2-Phenylphenol (incl. sodium salt orthophenyl phenol) **can be renewed under Regulation (EC) No 1107/2009**

It is considered that the following is specified in Part A of the Commission Implementing Regulation for the approval of the active substance:

Only uses as post harvest treatment may be authorised.

It is considered that the following be specified in Part B of the Commission Implementing Regulation as areas requiring particular attention from Member States when evaluating applications for product authorisation(s):

It is considered that it should be specified that conditions of use shall include risk mitigation measures, where appropriate.

It is proposed that the Member States concerned shall request the submission of confirmatory information:
(a) where new data requirements are established during the evaluation process, or
(b) as a result of new scientific and technical knowledge, or
(c) to increase confidence in the decision.

3.3 RATIONAL FOR THE CONDITIONS AND RESTRICTIONS TO BE ASSOCIATED WITH THE APPROVAL OR AUTHORISATION(S), AS APPROPRIATE

3.3.1 Particular conditions proposed to be taken into account to manage the risks identified

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
	[specify if measure relates to a specific representative use/use scenario/product or to all uses/products]

3.4 APPENDICES

GUIDANCE DOCUMENTS USED IN THIS ASSESSEMENT

General

- COMMISSION IMPLEMENTING REGULATION (EU) No. 844/2012 of 18 September 2012; setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No. 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.
- COMMISSION REGULATION (EU) No. 283/2013 of 1 March 2013; setting out the data requirements for active substances, in accordance with Regulation (EC) No. 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products in the market.
- COMMISSION REGULATION (EU) No. 544/2011 of 10 June 2011, implementing Regulation (EC) No. 1107/2009 of the European Parliament and of the Council as regards of data requirements for active substances.
- REGULATION (EC) No. 1907/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 December 2006. Concerning the Registration, Evaluation, Authorisation and Restrictions of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No. 793/93 and Commission Regulation (EC) No. 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.
- REGULATION (EC) No. 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008; on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No. 1907/2006.

Section identity, physical chemical and analytical methods

Section identity, physico chemical properties

- Manual on development and use of FAO and WHO specifications for pesticides: PLANT PRODUCTION AND PROTECTION PAPER 228; FAO/WHO Joint Meeting on Pesticide Specifications (JMPS); First edition-third revision; 2016.
- COUNCIL REGULATION (EC) No. 440/2008 of 30 May 2008
  Laying down test methods pursuant to Regulation (EC) No. 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)
- OECD Test Guideline 101: UV-VIS Absorption Spectra (Spectrometric Method), 12 May 1981
- OECD Test Guideline 102: Melting Point/Melting Range, 27 July 1995
- OECD Test Guideline 103: Boiling Point, 27 July 1995
- OECD Test Guideline 104: Vapour Pressure, 23 March 2006
- OECD Test Guideline 105: Water Solubility, 27 July 1995
- OECD Test Guideline 107: Partition Coefficient (n-octanol/water): Shake Flask Method, 27 July 1995
- OECD Test Guideline 109: Density of Liquids and Solids, 02 October 2012
- OECD Test Guideline 112: Dissociation Constants in Water (Titration Method), 12 May 1981
- OECD Test Guideline 115: Surface Tension of Aqueous Solutions, 27 July 1995
- UN Test N.4: Test method for self-heating substances. Classification Procedures, test methods and criteria relating to class 2, class 3, class 4, division 5.1, class 8 and class 9. United Nations, 2009.
- CIPAC MT 157: Water solubility, CIPAC Handbook 2009
- CIPAC MT 181: Solubility in Organic Solvents; CIPAC Handbook 2009

Section analytical methods

- SANCO 3030/99 rev. 5 of 22 March 2019: Technical Active Substance and Plant protection products: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex (Section 4) of Regulation (EU) No 283/2013 and Annex (Section 5) of Regulation (EU) No. 284/2013.
- SANCO 3029/ 99 rev. 4 of 11/07/00. Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414.
- SANCO /825/00 rev. 8.1 of 16/11/2010. Guidance document on pesticide residue analytical methods.
- SANTE 2017/10632 Rev. 3 of 22 November 2017: Technical Guideline on the Evaluation of Extraction Efficiency of Residue Analytical Methods.

Section Data on application and efficacy

Section Toxicology

Section Residue and consumer risk assessment

Section fate and behavior in environment

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FOCUS 2001. FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC. Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp., as updated by the Generic Guidance for FOCUS surface water scenarios, version 1.1 dated March 2012

FOCUS 2006. Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp. June 2006.

FOCUS 2008. “Pesticides in Air: Considerations for Exposure Assessment”. Report of the FOCUS Working Group on Pesticides in Air, EC Document Reference SANCO/10553/2006 Rev 2 June 2008. 327 pp.

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FOCUS 2014c. Generic guidance for Tier 1 FOCUS ground water assessments. Technical Report Version 2.2, FOrum for the Co-ordination of pesticide fate models and their Use

FOCUS 2015. Generic guidance for FOCUS surface water scenarios. Technical Report Version 1.4, FOrum for the Co-ordination of pesticide fate models and their USe. May 2015.

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Section ecotoxicology

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SETAC (Society of Environmental Toxicology and Chemistry), 2001. Guidance Document on Regulatory Testing and Risk Assessment procedures for Plant Protection Products with Non-Target Arthropods. ESCORT 2.

3.5 REFERENCE LIST

Section identity, physical chemical and analytical methods

- Monograph of 2-phenylphenol (2008).
- Confirmatory data of 2-phenylphenol, (2011).
- Review Report for the active substance 2-phenylphenol (SANCO/10698/09)
- Conclusion on pesticide peer review regarding the risk assessment of the active substance 2-phenylphenol (10.2903/j.efsa.2009.217r).
- https://echa.europa.eu/information-on-chemicals

Section data on application and efficacy

Section toxicology

Section residue and consumer risk assessment

- Monograph of 2-phenylphenol (2008).
- Confirmatory data of 2-phenylphenol, (2011).
- Review Report for the active substance 2-phenylphenol (SANCO/10698/09)
- Conclusion on pesticide peer review regarding the risk assessment of the active substance 2-phenylphenol (10.2903/j.efsa.2009.217r).

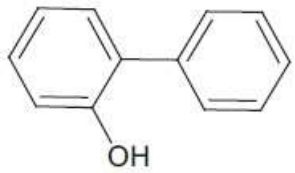
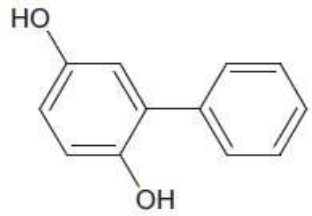
Section fate and behavior in environment

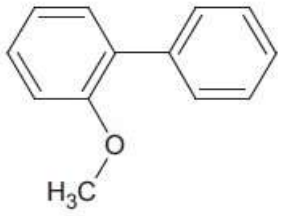
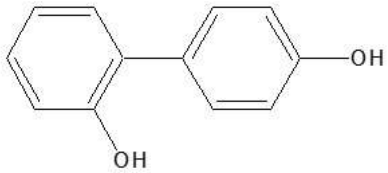
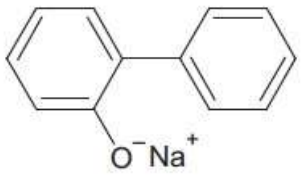
- Monograph of 2-phenylphenol (2008)
- Review Report for the active substance 2-phenylphenol (SANCO/10698/09)
- Conclusion on pesticide peer review regarding the risk assessment of the active substance 2-phenylphenol (10.2903/j.efsa.2009.217r)

Section ecotoxicology

- Monograph of 2-phenylphenol (2008)
- Review Report for the active substance 2-phenylphenol (SANCO/10698/09)
- Conclusion on pesticide peer review regarding the risk assessment of the active substance 2-phenylphenol (10.2903/j.efsa.2009.217r)

3.6 SUBSTANCES AND METABOLITES; STRUCTURES, CODES, SYNONYMS

Substance common name **Structure** **IUPAC name** **CAS name** **[CAS registry number]** **[EC number]**	**Molecular formula** **Molar mass** **Other names/codes**	**Occurrence**
2-Phenylphenol  Biphenyl-2-ol [1,1'-Biphenyl]-2-ol [90-43-7] [201-993-5]	C12H10O 170.2 g/mol Ortho-phenylphenol OPP	Parent substance used as test material Sulphate and glucuronide conjugates found in rodents and humans
2-Phenylhydroquinone  Biphenyl-2,5-diol [1,1'-Biphenyl]-2,5-diol [1079-21-6] [214-091-1]	C12H10O2 186.2 g/mol PHQ	Citrus Sulphate and glucuronide conjugates found in rodents and humans

Substance common name **Structure** **IUPAC name** **CAS name** **[CAS registry number]** **[EC number]**	**Molecular formula** **Molar mass** **Other names/codes**	**Occurrence**
2-Methoxybiphenyl  1-methoxy-2-phenylbenzene [1,1'-Biphenyl]-2-methoxy [86-26-0] [201-659-9]	C13H12O 184.2 g/mol 2-Phenylanisole 2-MBP	Citrus
2,4'-dihydroxy-biphenyl  2-(4-hydroxyphenyl)phenol [611-62-1]	C12H10O2 186.2 g/mol 2,4'-biphenol DHB	Sulphated conjugate found in rodents and humans
Sodium salt orthophenyl phenol  Sodium biphenyl-2-olate [1,1'-Biphenyl]-2-ol, sodium salt [132-27-4] or [6152-33-6] [205-055-6]	$C_{12}H_9NaO$ 192.2 g/mol Sodium-2-biphenylate SOPP	Parent substance